THE USE OF HISTOLOGICAL EXAMINATION METHODS TO DISTINGUISH BETWEEN THE BURNED REMAINS OF HUMAN AND NON-HUMAN BONES

By

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DECLARATION

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ABSTRACT

Distinguishing between burnt human and non-human bone fragments using macroscopic methods has proved challenging and it was seen in the literature review that the previous research did not all come to the same conclusions. The aim of the research was to determine if, using histological methods, it was possible to distinguish between burned human and non-human bone fragments. A literature review was compiled to provide an overview of the anatomy of bones, morphological characteristics of bone, general bone histology, a comparison between human and non-human bones and the effect of temperature on bones. Bones of five different species (human, baboon, wildebeest, pig and cow) were burned in a muffle furnace for twenty minutes at either 600°C or 800°C. Following the burning procedure, thin ground bone sections of the burned and unburned bone specimens were prepared for microscopic analysis and the minimum canal diameter, maximum canal diameter, minimum Haversian system diameter, maximum Haversian system diameter, area of canal, and area of the Haversian system were measured. A comparative analysis was then done across species and temperature. A total of 523 osteons in unburned bone and 147 in the burned bone samples were analysed. ANOVA testing found overall significance ($p < 0.0001$) for all parameters measured, which suggests that temperature does affect the size of micro-structures. *Most parameter sizes increased with an increase in temperature. A greater increase was seen at 600 degrees than 800 degrees.* Qualitatively, carbonation within the burned bone, made the measurement of parameters difficult in some samples. Human bone can easily be differentiated from pig, cow or wildebeest bone due to no or very few osteons present. Pig bone consisted almost entirely of plexiform bone, while the cow and wildebeest presented with only a few osteons in some parts of the bone. Human and baboon bone appeared similar on a microscopic level. The study revealed that temperature did not, in general, hamper the ability to differentiate between burned human and non-human bone, but it did impact on the number of measurable data points for each parameter.

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CHAPTER ONE – LITERATURE REVIEW

1. INTRODUCTION

Forensic science plays a crucial role in the resolution of crime, especially serious crimes that result in the death of a human being. In many cases all that is recovered is the bones of the victim, and it is often the role of the forensic anthropologist to make sense of what is found. Extended exposure of the human body to fire, leads to severe alterations especially in the head of the body. The alterations make it difficult to determine the cause of death and if a criminal element was involved. If the person was alive when the fire was started, the pathologist will see presence of soot in the airways, oesophagus and stomach on autopsy. Positive levels of carbon monoxide or cyanide may also be found depending on the fuel used and the nature of the fire (Fanton *et al*., 2006). Burning causes the bone to change both physically and chemically, which further causes difficulties in forensic identification tests. Heat-induced shrinkage can cause deformation and fragmentation, which will make the anthropometric analysis of species, sex, age and stature estimation difficult. Chemical modification of the bones also takes place due to burning. Combustion and pyrolysis of the chemical substances in the bone takes place. The severity of the degradation of DNA increases as the temperature rises, which will further compromise forensic identification techniques (Imaizumi, 2015). Human and non-human bone differs macroscopically, making it possible to distinguish between the two. Issues arise, however when highly fragmented bone is recovered. In such cases macroscopic differentiation is not possible and bones must be differentiated by other means. In certain instances, the death is as a result of fire, or fire is used as a means to attempt to dispose of the body or destroy evidence. In the event that only highly fragmented burned bone is found, there is a challenge in being able to determine, whether the bone fragments are of human origin or of some other species.

One method of determining the origin of bone fragments is through the microscopic examination of the bone. Human and non-human bone may contain the same features at a microscopic level, but factors such as pattern and size of structures can be used to make the differentiation. The presence of plexiform bone together with small secondary osteons and Haversian canals are indicative of non-human bone. Qualitative and quantitative differentiating factors must be used in conjunction to assess the origin of the unknown bone sample. Burning of bone can change the histological appearance of bone significantly.

2. GROSS ANATOMY OF BONES

Human skeleton bones can be grouped into basic but overlapping shapes. The bones found in the limbs consists mainly of long cylindrical shapes with expanding ends. Some of the hand and foot bones have a similar shape. Flat and cylindrical shaped bones can be found in the shoulder, pelvis, cranial vaulted and the rib cage. Irregular and block-like bones are found in the spine, wrist and ankle. Even though the bones in the human skeleton have different shapes externally they have a very similar makeup at both gross and microscopic levels (White *et al*., 2012).

All the bones of the adult skeleton have two basic structural components: spongy bone and compact bone. The shafts of long bones are made up of an outer layer (cortex) of cortical bone and an inner layer of trabecular bone. Compact or cortical bone is dense bone with a bony matrix filled with organic ground substances and inorganic salts, with only small spaces (lacunae) which contains the osteocytes. Compact bone comprises 80% of the human skeleton. Cancellous bone makes up the remaining 20% of the human skeleton. Compact bone is also the main component of the long bones. Mature compact bone is layered or lamellar in structure. The layered structure is permeated by the Haversian system, containing the blood supply for the osteocytes (Augustyn, 2020). Spongy bone (also called cancellous or trabecular bone) is more porous than compact bone and is predominantly found in the epiphysis (head) of long bones or sandwiched between flat bones. Spongy bone is made up of interconnected bony spicules called trabeculae. The long bone shaft (diaphysis) develops from the primary ossification centre of the bone. The primary ossification

centre assists the long bone in growing in length and appears before birth. All living bone is covered by the periosteum on the outside which is a vascular membrane that nourishes the bone. The endosteum is the cellular membrane that covers the inside surface of bones. Both these membranes are osteogenic tissues that contain bone-forming cells that are more active during the youth years than in adulthood (White *et al*. , 2012).

3. MORPHOLOGICAL CHARACTERISTICS OF BONE

Bone is a specialised connective tissue made up of bone cells and extracellular matrix. The extracellular matrix has three main constituents: an organic component, an inorganic component and water . Collagen comprises the main structural component of all connective tissue and it is also found in interstitual tissues of almost all parenchymal organs to assist with stability and structural integrity. Furthermore degradation of collagen and also disturbed metabolism of collagen forms part of osteoporosis and osteoarthritis development (Gelse *et al*., 2003).

Collagen is a general term used to describe proteins forming a triple helix of three polypeptide chains. All collagens has this supramolecular structures in the extracellular matrix, even though their size, function and tissue distribution varies a lot. Collagens can be grouped into fibril-forming collagens, fibril-associated collagens (FACIT), anchoring fibrils, transmembrane collagens, network-forming collagens, basement membrane collagens and others with specific functions.

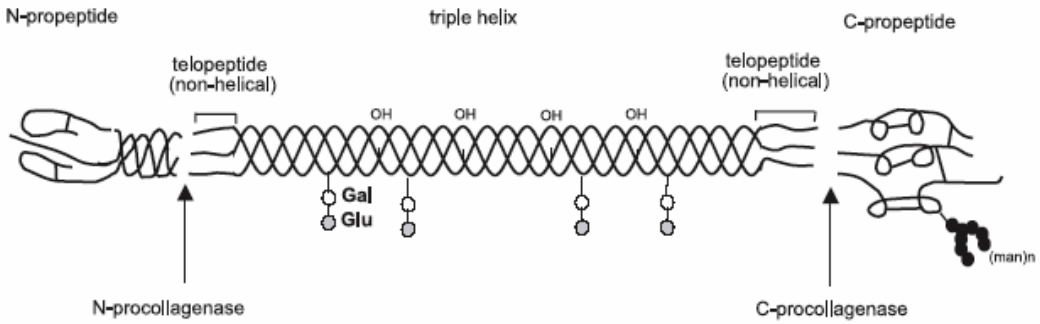


Figure 1 – Type I collagen molecule (Gelse et al., 2003)

Type I collagen is the collagen most studied and appears most often. Make up 90% of the organic mass of bone. Also the collagen present mainly in tendons, skin, cornea, ligaments and most interstitial connective tissues.

The exceptions to this is brain, vitreous body and hyaline cartilage. The triple helix of type I collagen is formed when two identical alpha 1 chains and one alpha 2 chain forms a heterotrimer. The type I collagen in bone defines biomechanical properties with regard to load bearing, tensile strength and torsional stiffness especially after calcification have taken place (Gelse *et al*., 2003).

The human skeleton consists of more than two hundred bones. The mineral found in the bones is a calcium phosphate, which resembles hydroxylapatite. It is called bioapatite because it has a close association with biological activity and molecules. Bioapatite may also be found in other tissues apart from bone and teeth as part of a pathological process. It may be found in tumours, due to the rapid cell production and cell death which take place in tumours. On cell death the phosphate bound to bio-organic molecules in the cell releases into the surrounding fluid. When this happen the calcium concentration in the circulating serum elevates above the calcium concentration in the cell and bioapatite may nucleate. Mineralization of bioapatite may also happen in scar tissue, due to tissue trauma, which lead to accumulation of excessive amounts of fibrous protein collagen as part of the healing process in the body. Fibroblasts are the cells which produces collagen. In bone tissue these cells are known as osteoblasts (Skinner, 2013).

All apatite minerals' crystal structure is dominated by tetrahedral anions. Phosphate in the phosphorous-oxygen tetrahedral anionic groups $(PO_4)^{3-}$ formation form the backbone of the structure and bond mainly to calcium. Bioapatite analysis is not always uniform in composition, because the mineral precipitate change over time. The phosphate ions ($H_2PO_2^{2-}$ and HPO_4) are usually detected in the fluid phase during initial mineral nucleation (Skinner, 2013).

Bone is made up of a hierarchical structure (Rho *et al*., 1998) ranging from the macroscopic level to the sub-nano scale (Figure 1). This hierarchical structure provides bone with its strength.

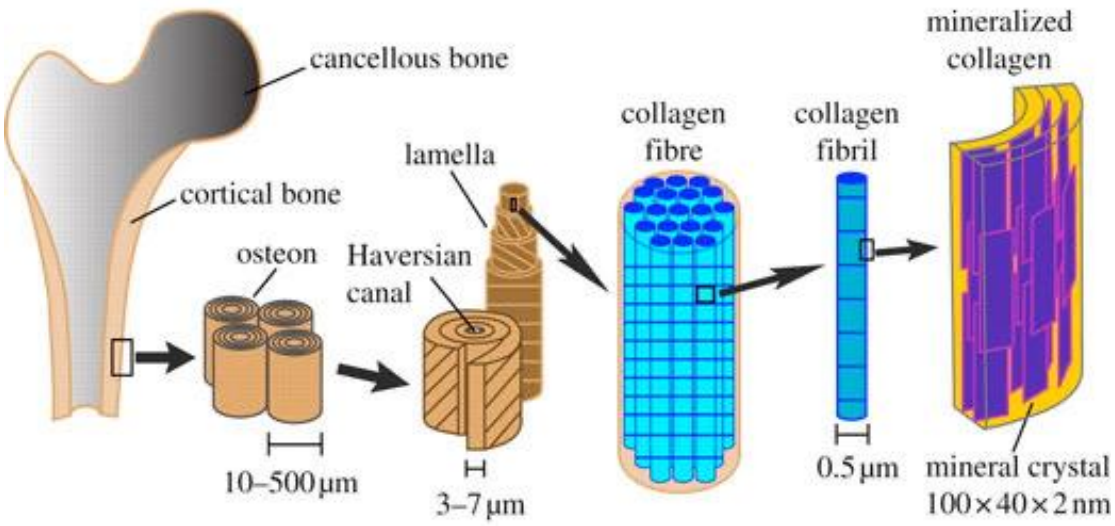


Figure 2 - Hierarchical Structure of Bone (https://www.nanowerk.com/news/newsid=22731.php)

The hierarchical organisation of lamellar bone consists of seven levels, where as woven bone can be divided into fewer levels.

Level 1: Major components

Similar material are present in both the ordered and disordered materials in the biomolecular and mineral components. Ordered material consists of type I collagen, carbonated hydroxyapatite and water with small amounts of other collagens, non-collagenous proteins (NCPs) and proteoglycans (GAGs). Disordered material consists of carbonated hydroxyapatite, type I collagen and "ground mass". The "ground mass" consists mainly of non-collagenous proteins, GAGs and water (Reznikov *et al.*, 2014).

Level 2: Structural components

In this level the collagen is organised into oval shaped fibrils. The gaps between the fibrils are filled with crystals of carbonated hydroxyapatite which extends into the overlap zones. This forms mineralised collagen fibrils. In this level there are also disordered materials present. They consists of mineralised collagen fibrils, ground mass and canaliculi (Reznikov *et al.*, 2014).

Level 3: Arrays

In this level the ordered material consists of arrays of self-assembling type I collagen fibrils. The disordered material consists of individual fibrils which are laid down in a disorganised manner (Reznikov *et al*., 2014).

Level 4: Array patterns

First pattern found is the unidirectional array pattern in which the fibril axes are aligned in larger dimensions. This pattern is commonly found in the following: lamellar bone, parallel fibered bone, mineralised tendons, Sharpey's fibres and other bone types.

Second pattern present at this level is the fanning array. In this array the fibril array changes in a gradational manner, which is also known as a twisted plywood pattern. This pattern is often seen in lamellar bone.

Thin layers of disordered material in found between the aligned collagen fibrils (Reznikov *et al*., 2014).

Level 5: Super-structure

The ordered material of this level consists of unidirectional collagen fibril bundles. This organised material is seen in human lamellar bone and mini-pig fibrolamellar bone, but it is absent in rat lamellar bone. Thin layers of disordered material containing the osteocyte process is present in human bone (Reznikov *et al*., 2014).

Level 6: Material patterns

At this level the tissues/materials of which an actual bone is made up of will be discussed. These tissues are the end-product of cellular activities. The most common bone tissue is plexiform bone (fibrolamellar bone), which consists mainly of parallel fibered bone which are found in rapidly growing animals. Woven bone is a type of bone frequently found in developing and also during fracture repair. Lamellar bone consists of both ordered and disordered material in each lamellae. Circumferential lamellar bone (CLB) consists of a group of lamellae with a large radius of curvature which are found close parallel to the forming surfaces of both compact and trabecular bone. The lamellae are laid down by the osteoblasts found on the bone surface. This bone is later replaced by secondary osteonal bone (Reznikov *et al*., 2014).

Level 7: Tissue elements

Lamellar bone is the main structural component in mammalian bone. Lamellar bone also replaces woven and fibrolamellar bone during remodelling. In spongy bone we find “lamellar packets”, which is a group of differently orientated lamellae. Primary and secondary osteons (Haversian systems) are tissue elements found in bone. In large animals mature skeletons, secondary osteons are mainly found since they are the product of remodelling. Fibrolamellar bone consists of a primary hypercalcified layer, made up of mineralised collagen fibril bundles. Next thick layers of parallel fibered bone are deposited on both sides of the primary hypercalcified layer. Any remaining voids are filled with lamellar bone. These fibrils are aligned with the long axis of the bone, ensuring a strong bone structure. “Fibrolamellar bone also has a disordered material that fills in the spaces between the collagen bundles, and the canalicular network is embedded within this disordered material” (Reznikov *et al*., 2014).

3.1. Macrostructure

Bone tissue is very dynamic, because it allows for growth during development of an individual. The bone tissue is shaped by cells, which can be found inside the tissue itself. Because of this attribute the morphology of bones can be changed during the lifetime of the individual. The shape and size of bones can also vary dramatically between individuals. Three major factors lead to variation in human skeletal anatomy:

- Ontogeny (growth)
- Sex
- Geography or population-based

Bone is distinguished into two types at the macroscopic level: cortical (compact) and spongy (or trabecular) bone. The end of a long bone, on cross-section, has a dense cortical shell with a porous spongy interior (Rho *et al*., 1998).

Flat bones, such as the bones of the calvarium, have a sandwich structure, consisting of dense cortical layers on the outsides and a thin reinforcing spongy structure on the inside. Both types of bone can be distinguished by their degree of porosity or density,

but true differentiation takes place through histological evaluations. This can only be done through microscopy methods (Rho *et al*., 1998).

3.2. Microstructure

Currey (2003) noted that two factors are involved in the ability of a bone to function effectively under the load imposed on it: firstly, the properties of the bone material and secondly the arrangement of the material in space, e.g. size and shape of bone.

Mammalian bone presents in two main forms, lamellar and woven bone. Woven bone is laid down fast and the collagen consists of randomly oriented fine fibres, which become highly mineralised. Lamellar bone is laid down slower and have a more precise structure. The collagen and its minerals in lamellar bone are organised in the lamellar plane, often in a characteristic direction. The collagen consists of thicker bundles than in woven bone. The mature bones of birds, mammals and large dinosaurs is called fibrolamellar bone. Fibrolamellar (plexiform) bone is found in animals who grow very fast. It is formed when woven bone is laid down to form a scaffolding which will be filled in by lamellar bone over a longer period of time. This leads to alternating layers of woven and lamellar bone tissue wrapped around the whole bone. Fibrolamellar bone is stronger when it is exposed to a load along the grain, but is brittle and weak when loaded across the grain. Secondary remodelling may take place if the load direction changes during growth of fibrolamellar bone (Currey, 2003).

Blood channels, erosion cavities, osteocyte lacunae and canaliculi are all potential stress concentrators. These stress concentrators increase the local stress by a factor, SCF, compared with the stress at a distance from the concentrator. Bone external surfaces are usually smooth, without sharp-edged protuberances, except at their ends. The major blood vessel entering the cortex of the bone, usually enter at a small angle to the long axis of the bone. Other blood channels, follow a longitudinal, but spiralling path in the long bones. These two arrangements of blood channel cavities cause minimal stress-concentrating effect, due the forces being directed mainly along the length of the bone. Volkmann's canals are more problematic due to them running directly transvers in the bone, which causes a higher stress-concentrating effect. The

blood channels in plexiform bone are arranged in a two-dimensional anastomosing network, which make the stress-concentrating effect small for longitudinal loads but large for radial loads (Currey, 2003).

Currey (2012) noted that water might be the main "gluing" agent between collagen and the minerals in bones. Fractures occur when the hydroxyapatite mineral crystals slide along a layered water film, followed by rupture of the collagen crosslinks (Currey, 2012).

Bone is routinely exposed to different loads such as compression, shear, tension, bending and torsion during an individual's lifetime, which brings Wolff's Law to the forefront. Wolff's Law states that, "bone is deposited where it is needed and resorbed where it is not needed" (White *et al*., 2012).

Wolff's Law is important because the structure, shape and size of bone tissue can be altered over time due to the mechanical load applied to it. Wolff's Law is more specifically pointed towards the development of trabecular orientation in long bones. Which is also known as the "trajectorial theory". It is mainly expressed in the proximal femur (Ruff *et al.*, 2006).

Ruff *et al*. (2006) further explains that the bone modelling/remodelling process is actually based on strain and not stress on the bone tissue and acts through feedback loops (Figure 3).

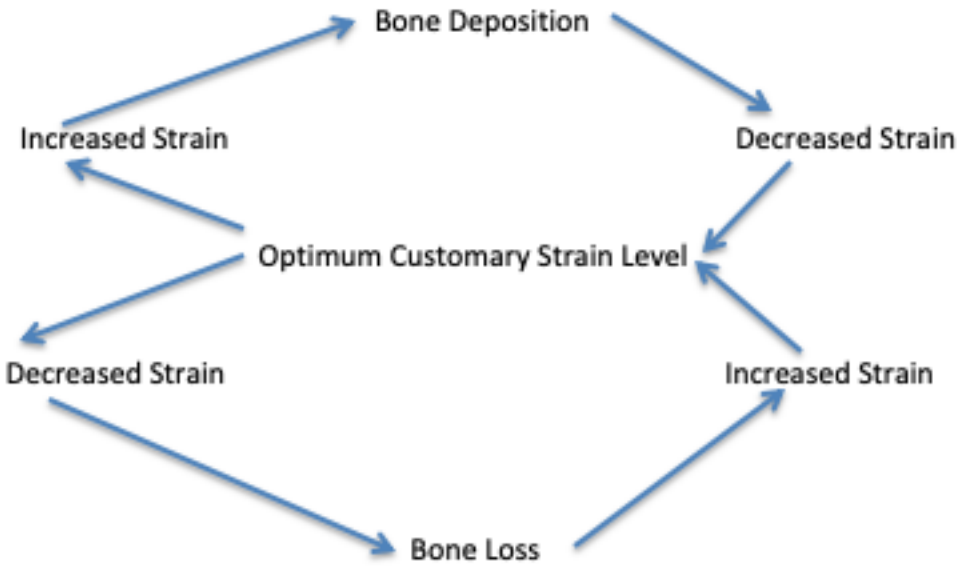


Figure 3 - Bone Modelling/Remodelling (Ruff et al., 2006)

Increase in activity level or increased strain leads to deposition of more bone tissue, which leads to reduction of strain to its original "optimum customary level". Inactivity or decreased strain on the other hand leads to resorption of bone tissue, which again restores the original strain levels (Ruff *et al*., 2006)

Bone is formed and maintained by means of three main types of cells. Osteoblasts deposits new bone material. When a bony matrix surrounds osteoblasts, they are known as osteocytes. Osteoclasts are involved in the resorption or removal of bone tissue (White *et al*., 2012).

Cross section of an adult long bone reveals the presence of circumferential lamellae bone at the periosteal and endosteal margins with the osteons scattered in between (Crescimanno & Stout, 2012).

Lamellae consists of mineralised collagen fibres which have formed into planar arrangements (3-7 µm wide) (Rho *et al*., 1998). Lucunae are the small cavities, which are found within each lamella. Inside each lacuna there is an osteocyte, a living bone cell. Canaliculi are very small fluid filled channels, which transport nutrients to the osteocytes (White *et al*., 2012).

A haversian system or an osteon is formed when lamellae wrap in concentric layers around a central canal. The osteons have the appearance of a cylinder about 200-250 µm in diameter. It runs more or less parallel to the long axis of the bone. Woven bone or immature bone is made up of mineral collagen fibres, which are less well registered, and no pattern can be seen. Lamellar bone is made up of lamellae and woven bone together, without osteon formation. The combination of bone forms a larger plywood-type stacking of layers (150-300 µm) around the circumference of the bone. Cancellous or spongy bone consists of an interconnecting framework of trabeculae in several different combinations. The combinations can be as follows: rod-rod, rod-plate or plate-plate. As noted above the rod's shape can also be referred to as a cylinder, thus the different trabeculae framework combinations can also be referred to as: cylinder-cylinder, cylinder-plate or plate-plate. The trabecular rod/cylinder has a diameter of 50-300 µm (Rho *et al*., 1998).

The nanoscale of bone consists of mineralised collagen fibres and the extrafibrillar minerals (Currey, 2012). Collagen fibres are made up of numerous fibrils approximately 0.5µm in diameter consisting predominantly of crystals (Rho *et al*., 1998).

4. GENERAL BONE HISTOLOGY

Human long bones in adults consists primarily of osteonic tissue.

Plexiform Bone

Plexiform bone is a primary bone tissue type (Figure 4) from the fibrolamellar bone tissue group. The cortical bone component of the long bones of fast growing animals, such as pigs, cows, dogs and other carnivores consists mainly of plexiform bone. Primates, including humans less frequently present with the presence of plexiform bone. The structure of plexiform bone is similar to laminar bone, but has a more dense system or plexus of vascularization. Longitudinal, radial and circumferential primary osteons make up a three-dimensional, symmetrical arranged network. Also seen in plexiform bone is rectilinear, residual vascular spaces, which result in the "brick wall" appearance of the bone type (Hillier & Bell, 2007).

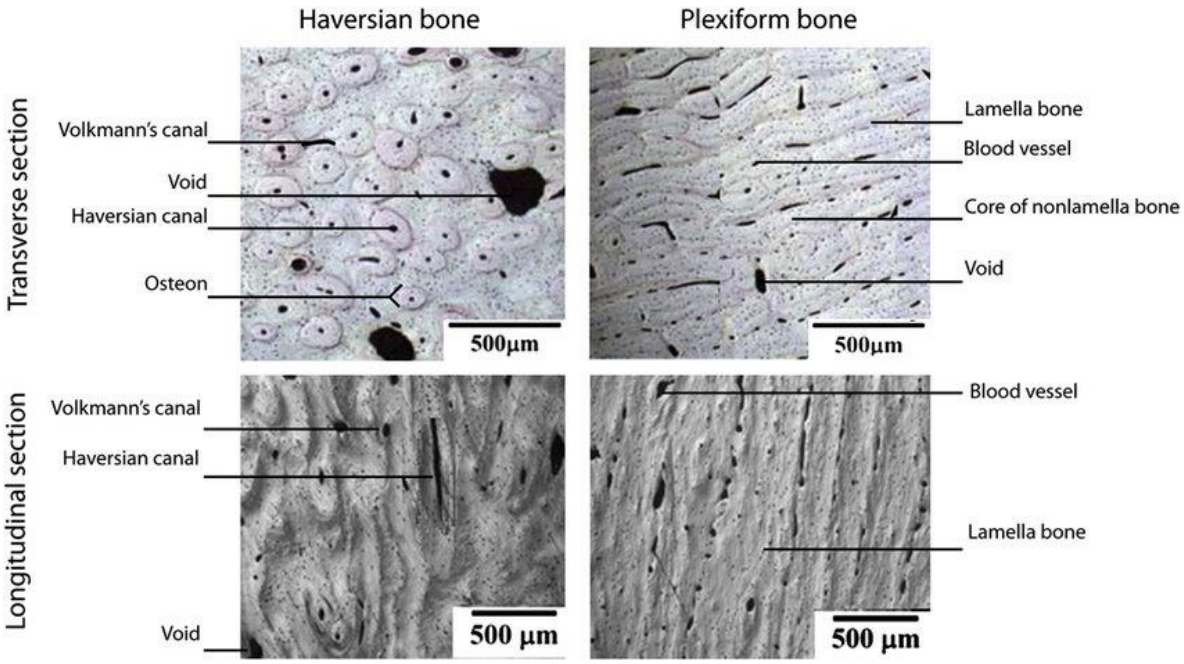


Figure 4 - Comparison between Haversian and Plexiform bone (https://www.researchgate.net/figure/Thin-section-comparison-of-Haversian-and-plexiform-bone-showing-diagnostic-structures_fig1_257154756)

Lamellar Bone

Lamellar bone is just one of several different types of bone. It is possible that lamellar bone carries out functions, which other members of the bone family and materials do not do. Lamellar bone is widely present in all classes of vertebrates. Lamellar bone is seen in mammals in load-bearing bones and in impact resisting bones. It has been said that lamellar bone might be the precursor of all bone types. Lamellar bone can be found in two basic forms: as extended parallel array lamellae (known as circumferential lamellar bone) or in cylinder form. The cylinder form is also known as osteons. The osteons fill up spaces left during the rapid growth of parallel-fibered bone. It forms a mixed type of bone, known as fibrolamellar bone. These osteons are elongated in shape and are called primary osteons. Secondary osteons are formed during the remodelling process of bone, after the removal of earlier formed bone. The secondary osteons are cylindrical in shape and are known as Haversian systems. Large animals tend to form bones which are made up of osteons in their entirety. The formation of secondary osteons takes place through a six-phase process. The phases are activation, resorption, reversal, formation, mineralization and quiescence (reversible growth arrest state). The process is achieved through osteoclasts and

osteoblasts working together in a complex arrangement. In the complex arrangement process the osteoclasts and osteoblasts are coordinated temporally and spatially to form the basic multicellular unit (BMU). The BMU consists of two separate components to form a three-dimensional structure. The two components present are a cutting cone and a closing cone. The cutting cone is lined with about ten osteoclasts and the closing cone is lined with hundreds of osteoblasts (Crescimanno & Stout, 2012).

The creation of the BMU is initiated during the activation phase. The process begins when the bone-lining cells withdraw and, in the process, expose the surface of the bone that will be resorbed. Cytokine secretion promotes osteoclast precursor cells to migrate to the area of exposed bone. They then develop into mature osteoclasts. These matured cells then resorb bone to form a bay or tunnel, which is known as a resorption bay or cutting cone. The cutting cone is 150-350µm in diameter in the exposed bone area. The cone "cuts" through the bone cortex at a rate of about 20µm/day in the direction of mechanical strain (Crescimanno & Stout, 2012).

After about three weeks the resorption phase is complete and then apoptosis of the osteoclasts occurs. After this happen the closing cone cells are attracted to the area of freshly resorbed surface. The next phase to follow is the reversal phase, during which the resorptive bay is smoothed out by a group of mononuclear cells. This forms a cement line or a reversal line. This line can be seen microscopically. The reversal phase takes about a month to complete and it represent the transition between osteoblast and osteoclast activity (Crescimanno & Stout, 2012).

The next phase is the formation phase, which lasts about three months. During this time the osteoblasts lining the closing cone are activated through several hormones and chemicals to form deposits of organic matrix known as osteoid. The osteoid comprises of type I collagen, proteoglycans, water and non-collagenous proteins. Over a period of 10-15 days the osteoid mineralizes and it forms concentric lamellae, through deposition of crystals of hydroxyapatite-like inorganic calcium phosphate. During the final phase the osteoblasts flatten into newly laid bone or they become trapped in the matrix, which they helped to form and then they are known as

osteocytes. The remodelling process takes about three to four months to complete, resulting in a bone structural unit (BSU). In cortical bone it is known as a secondary osteon (Crescimanno & Stout, 2012).

The smaller animals tend to keep the primary lamellar bone, which was laid down initially. When osteon concentrations are seen in smaller animals it is usually present in areas where tendons or ligaments attach to the bone (Weiner *et al*., 1999).

5. THE COMPARISON BETWEEN HUMAN AND NON-HUMAN BONES

A number of studies have been conducted which compare human and non-human bone. Macroscopically, complete non-human bones are often easily distinguished from complete human bones. However, when an analyst is presented with only highly fragmented pieces of bone, the differentiation becomes more complex. In such cases the use of histology has been found to be a cost effective and useful method (Cattaneo *et al*., 2009).

Histologically bones may differ qualitatively (shapes and patterns of microscopic structures) and quantitatively (metric measurements of microscopic structures). Qualitatively, one of the major findings is that many animal species have primarily plexiform bone (Mulhern & Ubelaker, 2001). However, a number of species exhibit a combination of plexiform and Haversian bone in different regions. If fragments are found containing only Haversian bone, the layout of the osteons may be used as a differentiating factor (Mulhern & Ubelaker, 2001). A number of non-human species (dog, horse, pig) exhibit banding (linear arrangement) of primary osteons. Furthermore, the shape/ geometry of the osteons may be used as a differentiating factor. It has been noted that human osteons are more elliptical in shape and non-human osteons are more circular in shape (Dominguez & Crowder, 2012). In contrast to these characteristics, adult human bone primarily consists of Haversian bone consisting of densely packed osteons randomly scattered throughout the bone. It has however been noted by both Andronowski, *et al*. and Mulhern and Ubelaker that osteon banding has been observed in human specimens (Mulhern & Ubelaker, 2001 ; Andronowski *et al*., 2017).

If osteon banding is not observed, then other characteristics must be looked for. The bone remodelling and deposition process are similar in non-human and human; thus, secondary osteons are a prevalent feature in bone microstructure (Dominguez & Crowder, 2012). In cases where secondary osteons are present in the bone tissue, the morphological characteristics of these structures may differ between species. Fragments of bone with Haversian systems, without the previously mentioned features can be difficult to differentiate between human and non-human bone. For this reason, there has been a move away from purely qualitative analysis to the development of more quantitative methods to analyse Haversian bone (Dominguez & Crowder, 2012).

During early research the main focus was the size differences of the microstructures of human and non-human bone. This did not lead to any broadly applicable methods to identifying human and non-human material. Measuring Haversian canal perimeter and secondary osteon size during research showed that osteon size varied across species and also identified the presence of age-related changes in Haversian canal size (Dominguez & Crowder, 2012).During studies which were done looking at osteon geometry it became clear that shape differences, and in particular osteon circularity can be potentially used to distinguish different species from one another. This characteristic can therefore potentially be used to differentiate Haversian bone in different species (Dominguez & Crowder, 2012).

Following is a description of the qualitative and quantitative characteristics of bone structure in species relevant to the current study. A thorough discussion of other species is beyond the scope of this study and the reader is referred to the following texts for further discussion relating to other species: Mulhern and Ubelaker (2012); Hillier and Bell (2007); Brits *et al*., (2014).

5.1. Qualitative Differences

5.1.1. Species Specific Bone Structure

5.1.1.1. Pig (Sus scrofa)

The two main histological features of pig bone is the presence of plexiform bone and osteon banding. These features make pig bone easily distinguishable from human bone (Crescimanno & Stout, 2012).

The femur of mature pigs consists of plexiform bone with dense haversian bone seen at the posterior area of the bone. The haversian canals, which are present, are medium in size (Hillier & Bell, 2007).

Martiniakova et al. (2007) reported the microscopic structure of the pig femur as mainly composed of primary vascular laminar bone tissue. The lamellae tissue is arranged in broad circumferential strata. Some areas in the femur show primary vascular canals organised into a well-defined plexus. In the anterior portion of the pig bone tissue there is dense haversian bone tissue, with a dense concentration of secondary osteons present (Martiniakova, *et al*., 2007).

In the forensic aspect of identification of bone, the area from where the fragment of bone is from can make the distinction between species difficult.

5.1.1.2. Sheep (Ovis aries)

The long bones in mature sheep consists of both plexiform and haversian bone tissue. Immature sheep have plexiform bone throughout the entire femur. There are a few haversian systems scattered in the posterior section of the bone (Hillier & Bell, 2007).

Martiniakova *et al*. (2007) reported that the haversian systems were more specifically located at the periosteal and endosteal borders (mainly antero-lateral view). The middle part of the compact bone shows dense haversian bone tissue.

5.1.1.3. Cow (Bos taurus)

Due to the butchering practice to slaughter sub-adult cows (13-24 months) there is no information available on the histology of adult cows. Sub-adult cow femur consists of plexiform bone at the endosteal surface, with a middle portion of laminar bone with an irregular arrangement. The periosteal region of the bone consists of haversian bone (Hillier & Bell, 2007).

5.1.1.4. Old World Monkeys (Ceropithecidae)

Included in this family are the baboons and macaques. The long bones of mature animals consist of dense haversian bone, with thin layers of endosteal and periosteal circumferential lamellae (Hillier & Bell, 2007).

Brits *et al*. (2014) did a study to find a tool to identify the differences between humans and animals mainly found in South Africa. They studied the periosteal surface of the femurs of monkeys, impala, cat, dog, cow, sheep, equid and pig. Human femurs were included as a comparative factor. The areas studied included only arrangement of vascular canals, primary osteons and secondary osteons. The results concluded that impala bone consists mainly of primary vascular longitudinal bone. It was also found that there is a degree of overlap and combinations of bone types, which lead to similar taxonomic orders being grouped together. Artiodactyls (pig, cow, sheep and impala) showed primary vascular bone. Carnivores such as cats and dogs as well as Penssodactyla (horses and donkeys) and primates all showed haversian bone (Brits *et al*., 2014).

5.2. Quantitative Differences

A number of quantitative differences have been shown to exist in the bone structure between different species. These variables include secondary osteon density, size of structures (Haversian canal or secondary osteon diameter, perimeter or area) as well as osteon shape or circularity. These differences are important in differentiating human and non-human bone. Nevertheless, previous studies have noted difficulties in such evaluations. This is due to studies having small sample sizes and varying ages of specimens used. Discriminant analysis procedures have been used more frequently in the last couple of years to differentiate between human and non-human bone. These studies used specifically the dimension of the Haversian canal size (Mulhern & Ubelaker, 2012).

Jowsey (1966) did a study of haversian systems in man and some animals. The animals which were compared to human included: rat, rabbit, cat, dog, Rhesus monkey, adult cow, Diadectes (extinct genus of large, reptile-like amphibians which lived during the

early Permian era) and Iguanodon (a type of ornithopod dinosaur). During this study the size of the osteons and the haversian canal perimeter were compared. It was found that the smaller the animal the smaller the size of the osteon and its canal. In the animals larger than a monkey, the size values remain constant with increasing body size. In the human specimens which were obtained from cortical bone in the rib and femur, it was found that in the femur the osteon size does not change much with age, however, the haversian canal perimeter size in the femur increases significantly with age ($P<0.001$). The rib haversian canals remain constant throughout life (Jowsey, 1966).

Saulsman *et al*., (2010) did a comparative study between human and the following animals to determine if quantitative morphometry can be used to differentiate between them if other morphological landmarks were absent: feral pig (Sus scrofa); sheep (Ovis aries); dog (Canis famillians); kangaroo (Macropus fuliginosus) and emu (Dromaius novaehollandiae). The sample size measured consisted of at least ten long bones from the different animal species and humeri, femora and tibia from fifty adult humans. Nonhuman primates have long bones, which are more elongated than in other mammals, when comparisons controlling for body mass were made. Nonhuman primates have greater midpoint transverse diameters at mid-length of the femur and the humerus, than other mammals. The sagittal diameter at the femur mid-length of nonhuman primates are greater than in other mammals. The head diameter of the humerus and femur are also greater in nonhuman primates than all other mammals (Kimura, 1995)

Urbanova and Novotny (2005) developed an identification key for distinguishing between human and non-human bone. Firstly, micrometric variables were examined using stepwise discriminant function analysis. Four equations were developed from this analysis wherein the following variables were found to be significant:

NO – number of osteons in $1mm^2$

DHC_{max} - maximum diameter of haversian canal

AHC – area of haversian canal

The relationship of the four equations to one another was used to successfully discriminate between human and non-human bone (94% classification accuracy). Including cortical thickness into the model improved the classification accuracy to 100%.

Crescimanno and Stout (2012) looked at using osteon circularity to differentiate between human and nonhuman bone samples. Circularity was calculated using the formula: Circularity = 4π (area/perimeter^2). The femora, humeri and ribs were used in the study. Using univariate ANOVA testing on pooled samples of the above mentioned bones in both human and non-human samples the results showed: F-statistic of 63.912 and a $p < 0.001$ which constituted a significant difference between the osteon circularity of pooled samples. It was found that the mean osteon circularity in human samples was 0.85 which is considerably lower than nonhuman circularity which was found to be 0.871. Using this method, they classified 76,5% of samples correctly (Crescimanno & Stout, 2012).

Dominguez and Crowder (2012) similarly used osteon circularity and osteon shape to differentiate between human and nonhuman haversian bone. They used dog and deer samples as the non-human component, because both are frequently recovered in both the forensic and archeological setting. Osteon shape/area showed a clearer delineation between human and nonhuman samples than osteon circularity. On average human osteons had a larger area than nonhuman osteons (deer and dog). Significant differences were established using osteon area alone, however, the success rate of differentiating human and nonhuman bone increased when osteon circularity was also taken into account (Dominguez & Crowder, 2012).

Human and non-human primates have similar structures histologically. Non-human primate femoral bone shows the presence of a combination of avascular bone and irregular haversian bone. The non-human bone has a few longitudinal vascular canals (Kimura, 1995). Nganvongpanit *et al*. (2015) worked on a species identification method through osteon structure of adult humans and Assam macaques . Humeri, radi, ulni, femurs, tibiae and fibuli were assessed during the study using histological methods. The measurements taken included:

- Diameter of haversian canal and osteon
- Perimeter of haversian canal and osteon
- Area of haversian canal and osteon
- Distance between centre of haversian canal
- Ratio between diameter of osteon and haversian canal

The outcome of the study was that the above mentioned parameters were significantly higher in all bones in humans except the diameter of the Haversian canal in the radii and the area of the Haversian canal in ulnae, which were higher in the Assam macaques than in humans (Nganvongpanit *et al*., 2015).

Martiniakova *et al*. (2007) assessed qualitative and quantitative characteristics of different species' bone. These characteristics included:

- Measured area, perimeter and min/max diameter of haversian canals and secondary osteons
- Vascular canals of primary osteons were also measured

They concluded that every species differed qualitatively from each other in bone tissue type or combinations of tissue types in different areas of thin sections viewed. When only qualitative aspects were considered for identification it was only 73,83% correct. Through adding quantitative analysis results the identification correctness can approach 100% (Martiniakova , *et al*., 2007).

The typical ranges for qualitative characteristics in human femur bones differ from study to study conducted. Minimum and maximum Haversian canal diameter varies from 20.6 µm to 77.4µm. Haversian canal perimeter varies from 35.84 µm to 173.0 µm. Haversian canal area varies from 1096.98 μm^2 to 2164.15 μm^2 . Osteon diameter varies from 19.19 µm to 263.76 µm. Osteon perimeter varies from 102.48 µm to 550.85 µm. Osteon area varies from 37762.06 μm^2 to 12860.20 μm^2 (Mulhern & Ubelaker, 2012). The structures within non-human bones will vary based on the type

of animal. For typical ranges seen in various animals the reader is referred to Mulhern and Ubelaker (2012).

6. THE EFFECT OF TEMPERATURE ON BONES

Forensic anthropology is involved in interpretation of burned bone. As evident above the differentiation of human from non-human bone using histological methods is well established. However, burning of bone can alter or destroy many of the structures previously discussed. There has been limited research investigating the extent of such alterations or what effect such alterations may have on the ability to differentiate between human and non-human bone.

Bodies are burned in a number of different circumstances which may be deliberate (homicide or in an attempt to destroy evidence) or accidental (industrial/ household fires, aircraft and road traffic accidents). Archeological bone samples may also present with evidence of burning. Burned bone can present as highly fragmented and may be comingled with debris and rubbish (which may include bones of non-human origin). Identification of the fragments as being human in origin can therefore be a difficult task (Ubelaker, 2009).

Reconstruction of burned bone samples, human or non-human is frequently overlooked. Reconstruction can help with the recognition of specific skeletal elements and trauma. Interpretation of trauma can be difficult due to the presence of heat related fractures or fragmentation. Disease and age at death can also influence analysis of burned bone samples. It was found in experimental research that osteoporotic bone fragments more frequently than healthy bone (Ubelaker, 2009).

It is documented that burning of fleshed remains produce distinctive transverse fractures in a curvilinear pattern, irregular longitudinal splitting and considerable warping. Burning of dry bone samples produce less variation in fracture patterns, longitudinal splitting and less warping (Ubelaker, 2009).

When looking at the effect of fire on bones and other tissue, it is not just the temperature, but also the duration of the heating which plays a role (Thompson, 2005). Bone as a living tissue contains blood, water, fats and other tissues in a

complicated matrix. When the bone is heated all these components react in different ways, such as evaporating, charring, liquefying, contracting and expanding (Schmidt & Symes, 2015).

Waterhouse (2013) describes the process, which human remains undergo when burned as follows: as the bone is exposed to the burning, the bone material changes from the natural state to the calcined state. The heat-induced changes, are changes in gross and microscopic appearance, size, colour and shape. As the bone burns it loses water and organic compounds.

The inorganic component of bone is a mineral known as bio-apatite or b-HAP, which is a poorly crystalline form of the mineral hydroxyapatite ($Ca_5(PO_4)_3(OH)$,HAP). It gets the poor crystalline properties from ion substitutions, ion vacancies in the b-HAP crystal lattice and through ion adsorptions on the crystal surfaces (Beckett *et al*., 2011).

This mineral is very important within forensic and archaeological contexts, since b-HAP is possibly the most significant surviving component of bone. Advances in analytical methods have led to the use of b-HAP as a possible species identification tool (Beckett *et al*., 2011).

Some changes in b-HAP, which can take place during heating of bone include:

- Increase in crystallinity, which is caused by an increase in crystal size and or a decrease in microstrain (lattice disorder).
- Ion substitutions and vacancies which can lead to lattice defects.
- Other or additional mineral phases can form at the expense of b-HAP (Beckett *et al*., 2011).

The structural integrity of bone alters as the overall size and shape of the bone changes. As the structural integrity is altered, the stress-strain relationship of the different bone elements is changed, leading to mechanical failure, which in the case of bone is a fracture. During the burn process the bone lose organic material, which leads to a reduction in bone elasticity, which further increase the fragmentation of

calcined material (Waterhouse, 2013). It is clear that bone undergoes a vast amount of changes. These changes, particularly on a microscopic level, need to be taken into account when attempting to determine the origin of burned bone fragments.

Bone dehydration takes place between 100°C and 300°C and it reduces the size of the piece of bone by one to two percent of the original volume (Castillo *et al*., 2013). The next phase in burning of bone is the decomposition phase, which is marked by the removal of organic components from the bone through pyrolysis. The third phase in burning of bone is the inversion phase, during which the bone loses carbonate. The final phase which bone undergoes during burning is the fusion phase, which is characterised by the melting and coalescing of the crystal matrix (Ellingham *et al*., 2015)

6.1 COLOUR CHANGE

The main macroscopic effect, which can be seen on burned bones, is colour change. The colour a heat exposed bone exhibit is dependent on the ratio of organic and inorganic components present in the bone. The colour a bone exhibit is also dependent on the temperature it was exposed to and the time it was exposed for. Ellingham *et al*. (2015) tabulated the temperatures and colour changes, which are summarised below:

Table 1 - Colour Changes in Burned Bone at Different Temperatures

Temperature (°C)	Colour observation
100 - 200	Yellowish
300 - 400	Shades of brown/grey/black
500 - 600	Grey/black
700 - 800	Shades of white/grey
900 - 1000	White

Several factors will determine which colour the heat-exposed bone will be after exposure. The colour changes the bone undergoes is dependent on the ratio between organic and inorganic components present in the bone and how these respond to the

temperature applied. Fresh bone is usually a light ivory colour, which turn from brown into black due to carbonization. During the next phase in the combustion process the organic components pyrolize, which causes a grey shading in the bone. Then the bone turns white, which shows calcination and complete loss of all organic compounds and fusion have taken place in the bone minerals (Ellingham *et al*., 2015).

These colour changes take place in bone as the bone dehydrates and also get exposed to the gradual loss and shrinkage of muscle tissue if the bone is still encased in flesh. Since the soft tissues are not the same thickness all over, the bone will not burn uniformly. Bones usually burn from the outside to the inside, which leads to the presence of lighter calcined bone on the outside surface and with black charred surface on the inside of the bone (Schmidt & Symes, 2015).

6.2. HEAT INDUCED FRACTURES

Burning of bones can also lead to different types of heat induced fractures. There are seven types of fractures as described by Schmidt and Symes (2015), which can be observed in burned bone.

Longitudinal

These types of fractures are commonly seen in long bones. They occur when the bone shaft is heated to the point of evaporation and protein denaturalization. The bone matrix then starts to shrink, which causes structural failure. The fractures tend to occur in similar areas of the bone following the grain of the bone and parallel to the osteon canals.

Step

These types of fractures are seen in conjunction with the longitudinal type. They extend from the longitudinal fracture margin, transversely across the bone shaft, through the compact bone, fracturing the bone shaft.

Transverse

The difference between longitudinal fractures and transverse fractures is that the transverse fractures transect the haversian canals. Transverse fractures are commonly seen in the long bones, since the fire consumes the long bones transversely as it travels up the bone shaft. Step fractures are also seen with transverse fractures.

Patina

This superficial fracture type can be described as craquelure as seen on old china or paintings. The result of a larger area of bone receiving a constant amount of heat during a fire or controlled burning. They are prevalent in flat areas of bone and postcranial bones, but can also be seen on epiphyseal ends and cranial bones.

Splintering and Delamination

The main characteristics of this type of fracture is the splitting away of cortical bone layers from the cancellous bone, the separation of the tables of the cranial bone, or the exposure of cancellous bone present on the epiphyses and also the costochondral rib ends.

Curved Transverse

These fractures result when the heated bone's soft tissue is burned away and the periosteum shrink, pulling the brittle surface of the burned bone. They can also be seen in concentric rings in the popliteal area of the femur, when the femur is burned.

6.3 THE MICROSCOPIC EFFECTS

Previous studies have indicated that the most important discriminating factor in the assessment of the human origin of a bone sample is related to the metrics of the Haversian canal. Changes in size and shape of these structures may therefore cause issues in differentiation between human and nonhuman particularly in cases where there is large overlap between species.

As noted by Ellingham *et al*. (2015) there are several studies which look at the microscopic changes, which take place in burned bone, but they do not reach consensus in the results obtained. An increase in osteon size, with increase in temperature was noted by Bradtmiller & Buiksta (1984). A decrease in osteon size but a minimal increase in Haversian canal diameter were observed by Forbes (1941), Nelson (1992) and Hummel & Schutkowski (1993). Absolonova *et al*. (2012) found in a study done on ribs heated to 700°C and 800°C that the osteon and Haversian canal size decreases. Therefore, further study of the changes bone undergoes when burned is needed (Ellingham *et al*., 2015).

Hanson and Cain (2007) did a study on histological changes in heated bone and found that if no or very little heat (0°C to 470°C) is applied to bone samples, there is no histological changes or cracking visible. Low to medium heat (± 590°C) also showed no cracking histologically. Medium heat (380°C to 482°C) produced cracks radiating from the haversian canals. Medium to high (482°C to 620°C) heat shows a similar outcome to the medium heat. High heat (462°C

to 705°C) presents with lost histological structure and wide cracks throughout the sample (Hanson & Cain, 2007).

Imaizumi *et al*. (2014) in a study investigating heat induced structural changes in bovine bones documented several changes, such as cracking and osteon separation due to the increase in temperature. The proportion of osteon separation and cracking was different at each temperature tested in their study and this can possibly be used to estimate the temperature at which the bones were burned. This feature can be helpful in dealing with forensic case studies (Imaizumi *et al*., 2014).

Minute fissures are seen in histological observations, but on the surface of burned bone there are also visible cracks seen. These cracks increase in severity together with the increase in the temperature of the burn. These cracks can furthermore obscure traumatic injuries, which are crucial for estimation of cause of death (Imaizumi *et al*., 2014).

7. CONCLUSION

Anthropologists have concerned themselves with the study of thermal alteration on human remains out of necessity; due to the impact fire and heat have on their analyses and interpretations. Valuable evidence can be destroyed through the destructive force of fire.

It is very important to distinguish between burned human and animal bones in the forensic science field. Whole bones can generally be identified through macro-morphological characteristics, but problems arise when bones are highly fragmented and damaged due to fire and thus need histological examination. The method used to determine the origin of the bones must be reliable and take the different factors such as temperature of the fire, type of bone structure and fracture types into consideration.

We can see that there are several different areas, which have been studied extensively. These include the colour changes in bones exposed to heat, the fracture patterns present after exposure to different temperatures and also the morphological differences present in animals and humans. It is however evident that there is a paucity of literature regarding the study of histological changes, which occur in burned human and animal bones.

Thus, the current study aims to determine the utility of histology to differentiate between different species femur bones in either a burned or unburned state.

8. LIST OF REFERENCES

Absolonova, K., Dobisikova, M., Beran, M., Zocova, J., & Veleminsky, P. (2012). The temperature of cremation and its effect on the microstructure of the human rib compact bone. *Anthropologischer Anzeiger*, 69(4), 439-460

Andronowski, J., Pratt, I., & Cooper, D. (2017). Occurance of osteon banding in adult human cortical bone. *American Journal of Physical Anthropology, 164*(3), 635-642

Augustyn, A. (2020, February 6). *britannica.com*. Retrieved from Encyclopaedia Britannica: https://www.britannica.com/science/compact-bone

Beckett, S., Rogers, K., & Clement, J. G. (2011). Inter-Species Variation in Bone Mineral Behaviour upon Heating. *Journal of Forensic Sciences, 56*(3), 571-579.

Bradtmiller, B., & Buiksta, J. (1984). Effects of burning on human bone microsctructure: a preliminary study. *Journal of Forensic Science, 29*(2), 535-540.

Brits, D., Steyn, M., & L' Abbe, E. N. (2014). A histomorphological analysis of human and non-human femora. *International Journal of legal medicine, 128*(2), 369-377.

Castillo, R. F., Ubelaker, D. H., Acosta, J. A., & Canadas de la Fuente, G. A. (2013). Effects of temperature on bone tissue. Histological study of changes in the bone matrix. *Forensic Science International, 226*(1), 33-37.

Cattaneo, C., Porta, D., Gibelli, D., & Gamba, C. (2009). Histological Determination of the Human Origin of Bone Fragments. *Journal of Forensic Sciences, 54*(3), 531-533.

Crescimanno, A., & Stout, S. D. (2012). Differentiating Fragmented Human and Nonhuman Long Bone Using Osteon Circularity. *Journal of Forensic Sciences, 57*(2), 287-294.

Currey, J. (2003). The many adaptations of bone. *Journal of Biomechanics, 36*(10), 1487-1495.

Currey, J. D. (2012). The structure and mechanics of bone. *Journal of Material Science, 47*(1), 41-54.

Dominguez, V. M., & Crowder, C. M. (2012). The Utility of Osteon Shape and Circularity for Differentiating Human and Non-human Haversian Bone. *American Journal of Physical Anthropology*(149), 84-91.

Ellingham, S. T., Thompson, T. J., Islam, M., & Gillian, T. (2015). Estimating temperature exposure of burnt bone - a methological review. *Science and Justice, 55*(3), 181-188.

Fanton, L., Jdeed, K., Tilhet-Coartet, S., & Malicier, D. (2006). Criminal burning. *Forensic Science International, 158*, 87-93.

Forbes, G. (1941). The effects of heat on the histological structure of bone. *The Police Journal, 14*(1), 50-60.

Gelse, K., Poschl, E., & Aigner, T. (2003). Collagens - structure, function, and biosynthesis. *Advanced Drug Delivery Reviews*(55), 1531 - 1546.

Hanson, M., & Cain, C. R. (2007). Examining histology to identify burned bone. *Journal of Archeological Sciences, 34*, 1902-1913.

Hillier, M. L., & Bell, L. S. (2007). Differentiating human bone from animal bone: A review of histological methods. *Journal of Forensic Science, 52*(2), 249-263.

Hummel, S., & Schutkowski, H. (1993). Approaches to the histological age determination of cremated human remains. In *Histology of ancient human bone: methods and diagnosis.* Berlin, Heidelberg: Springer.

Imaizumi, K. (2015). Forensic investigation of burnt human remains. *Research and Reports in Forensic Medical Science, 5*(67)

Jowsey, J. (1966). Studies of Haversian systems in man and some animals. *Journal of Anatomy, 100*(4), 857-864.

Kimura, T. (1995). Long bone characteristics of primates. *Zeitschrift fur Morphologie und Anthropologie, 80*(3), 265-280.

Martiniakova, M., Grosskopf, B., Omelka, R., Dammers, K., Vondrakova, M., & Bauerova, M. (2007). Histological Study of Compact Bone Tissue in Some Mammals: A Method for Species Determination. *International Journal of Osteoarchaeology, 17*(1), 82-90.

Mulhern, D. M., & Ubelaker, D. H. (2001). Differences in Osteon Banding Between Human and Nonhuman Bone. *Journal of Forensic Science, 46*(2), 220-222.

Mulhern, D. M., & Ubelaker, D. H. (2012). Differentiating Human from Nonhuman Bone Microstructure (Chapter 5). In C. Crowder, & S. Stout, *Bone Histology - An Anthropological Perspective* (pp. 109-134). Boca Raton: CRC Press.

Nelson, R. (1992). A Miscroscopic Comparison of Fresh and Burned Bone. *37 No 4*, 1055-1060.

Nganvongpanit, K., Phatsara, M., Settakorn, J., & Mahakkanukrauh, P. (2015). Differnces in compact bone tissue microscopic structure between adult humans (Homo sapiens) and Assam macaques (Macaca assamensis). *Forensic Science International*, 243.e1 - 243.e5.

Reznikov, N., Shahar, R., & Weiner, S. (2014). Bone hierarchical structure in three dimensions. *Acta Biomaterialia, 10*, 3815 - 3826.

Rho, J. Y., Kuhn-Spearing, L., & Zioupos, P. (1998). Mechanical properties and the hierarchical structure of bone. *Medical Engineering & Physics, 20*, 92-102.

Ruff, C., Holt, B., & Trinkaus, E. (2006). Who's Afraid of the Big Bad Wolff?: "Wolff's Law" and Bone Functional Adaptation. *American Journal of Physical Anthropology, 129*, 484-498.

Saulsman, B., Oxnard, C. E., & Franklin, D. (2010). Long bone morphometrics for human from non-human discrimination. *Forensic Science International*, 202(1-3), 110.e1 - 110.e5.

Schmidt, C. W., & Symes, S. A. (2015). *The analysis of burned human remains.* (2nd Edition ed.). Academic Press: London, United Kingdom

Skinner, H. (2013). Minerology of Bone. In O. Selinus, *Essentials of medical geology: Revised edition.* Elsevier.

Thompson, T. (2005). Heat-induced Dimensional Changes in Bone and their Consequences for Forensic Anthropology. *Journal of Forensic Science, 50*(5), 1-8.

Ubelaker, D. H. (2009). The forensic evaluation of burned skeletal remains: A synthesis. *Forensic Science International*, 1-5.

Urbanova, P., & Novotny, V. (2005). Distinguishing between human and non-human bones: histometric method for forensic anthropology. *Anthropologie*, 77-85

Walls, R., Olivier, G., & Eksteen, R. (2017). Informal settlement fires in South Africa: Fire engineering overview and ful-scale tests on "shacks". *Fire Safety Journal, 91*, 997-1006.

Waterhouse, K. (2013). Post-burning fragmentation of calcined bone: Implications for remains recovery from fatal fire scenes. *Journal of Forensic and Legal Medicine, 20*(8), 1112-1117.

Weiner, S., Traub, W., & Wagner, D. (1999). Lamellar Bone: Structure - Function Relations. *Journal of Structural Biology, 126*(3), 241-255.

White, T. D., Black, M. T., & Folkens, P. A. (2012). *Human Osteology* (Third ed.). Elsevier Academic Press: London.

CHAPTER TWO – JOURNAL ARTICLE

THE USE OF HISTOLOGICAL EXAMINATION METHODS TO DISTINGUISH BETWEEN THE BURNED REMAINS OF HUMAN AND NON-HUMAN BONES

ABSTRACT

Distinguishing between burned human and non-human bone fragments using macroscopic methods has proved challenging. The aim of the research was to determine if, using histological methods, it was possible to distinguish between burned human and non-human bone fragments. Bones of five different species (human, baboon, wildebeest, pig and cow) were burned in a muffle furnace for twenty minutes at either 600°C or 800°C. Following the burning procedure, thin ground bone sections of the burned and unburned bone specimens were prepared for microscopic analysis and the minimum canal diameter, maximum canal diameter, minimum Haversian system diameter, maximum Haversian system diameter, area of canal, and area of the Haversian system were measured. A comparative analysis was then done across species and temperature. A total of 523 osteons in unburned bone and 147 in the burned bone samples were analysed. ANOVA testing found overall significance ($p < 0.0001$) for all parameters measured, which suggests that temperature does affect the size of micro-structures. Most parameter sizes increased with an increase in temperature. A greater increase was seen at 600 degrees than 800 degrees. Qualitatively, carbonation within the burned bone, made the measurement of parameters difficult in some samples. Human bone with many osteons present can easily be differentiated from pig, cow or wildebeest bone due to no or very few osteons present in these animal bones. Pig bone consisted almost entirely of plexiform bone, while the cow and wildebeest presented with only a few osteons in some parts of the bone. Human and baboon bone appeared similar on a microscopic level. The study revealed that temperature did not, in general, hamper the ability to differentiate between burned human and non-human bone, but it did impact on the number of measurable data points for each parameter.

Keywords: Burned bone; Histology; Species differentiation

1. INTRODUCTION

The identification of burned bone obtained from a potential crime scene can be challenging, especially when one needs to determine whether the bone is of human or animal origin. Being able to distinguish between animal and human bones can be crucial in any forensic investigation, especially to determine whether a human victim is present on a potential crime scene. DNA testing and protein testing may not always be useful in identifying whether or not bone fragments can be linked to a particular species due to degradation (Cattaneo *et al*., 2009). When these methods are not suitable, then anthropological methods, such as bone histology, may be the only forensic examination option available.

Bone histology, which allows the examination of bone at a microscopic level, can play a role in the identification of bones from various species. Qualitative and quantitative differences in the histological structure of bone of various non-human species have been noted (Owsley *et al*., 1985). This can be achieved by examining the structure of the bone microscopically, for example, by examining plexiform bone structures (which are generally never observed in adult humans), or examining haversian bone structures, such as osteon morphology (Cattaneo *et al.*, 2009).

The microscopic examination of bone fragments can generally identify whether or not a bone fragment is likely to be from a human or other non-human species (Mulhurn and Ubelaker, 2012; Cattaneo *et al*., 2009) However, when one considers bone fragments that are burned, distinguishing between human and other non-human species becomes more difficult (Cattaneo *et al*., 1999; Ubelaker, 2009)

When bone is subjected to extreme temperatures in fires, the bone itself is altered on a macroscopic and microscopic level. This can make the macroscopic identification (such as through visual identification of the fragment) of burned bone fragments difficult (Beckett *et al*., 2011).

The majority of research into the histological examination of bone fragments to distinguish between human and other non-human species have focused on the examination of unburned bone (Cattaneo *et al.,* 1999) here has been limited research on the histological examination

of burned bone fragments to identify whether or not burned bone fragments can be identified as having come from human remains, or another non-human species.

Researchers such as Shipman *et al*. (1984), Thompson (2004, 2005), Holden *et al*. (1995), Cattaneo *et al*. (1999) and Hanson and Cain (2007) all identified different methods to use in distinguishing between burnt human and animal remains, using either macroscopic or microscopic forensic methodologies to examine the bones. Shipman *et al*. (1984) found that burial of burned bones alter the colour, thus the criteria from a controlled burn can not be used to determine the temperature the bones were burned at. They used a combination of colour, microscopic morphology and X-ray diffraction patterns to assist in identification if a skeleton was burned and if so, at what temperature. Thompson (2005) looked at heat-induced dimensional changes in bone and some of the consequences it cause for forensic anthropologists. Cattaneo *et al*. (1999) studied the use of histological, immunological and DNA techniques to determine the origin of bone fragments. They found that histological methods were most accurate in determining origin of bone, while immunological and DNA techniques were less accurate. Holden *et al*. (1995) looked at the use of scanning electron microscopy (SEM) to identify bone fragments from fire victims compared to samples burned in controlled laboratory setting. It was found that both settings produce similar features at an ultrastructural level. Hanson and Cain (2007) studied the use of histology to identify burned bone. They looked at modern burned bones compared to archaeological bones from Sibudu Cave (*ca*. 60 000 years ago) and Cave of Hearths (more than 200 000 years ago). It was found that open fires and controlled heating in an oven produce similar changes to the bone microstructure, with the difference that the open fire samples are not as uniform in the results.

Mohd Nor *et al*. (2013) stated that morphological skeletal features are frequently used in people younger than 50 years of age to estimate age at death. Histology is noted as the first choice in determining age in people over 50 years of age. Bone changes in morphological and histological features with age due to turnover, remodelling and modelling and growth. With age there is also increased bone porosity due to increased bone resorption and less formation. Influences that can have an impact on determining the rate of skeletal ageing include: poor nutrition, life history, disease, diet, physical activity and length of time spend in daylight (Mohd Nor *et al*., 2014).

Rate of development and bone histology have several differences among mammals. Many large mammals have plexiform bone due to rapid growth rate of juveniles. Primates develop haversian bone. During initial growth in primates lamellar bone, primary canals and primary osteons are formed. Later in the lifespan of the primates these are replaced by secondary osteons, depending on the mechanical and nutritional demands. Comparing humans and chimpanzees, the humans have an extended period of growth. Epiphyseal union also occur later in humans than chimpanzees. Comparing same age humans and chimpanzees, the age of the chimpanzee is about two-thirds that of the human.

Age at death in humans can be calculated based on the number of osteons present, due to the fact that osteons and osteon fragments accumulate with age. There is also a decrease in the amount of circumferential lamellar bone in older age (Mulhern & Ubelaker, 2003).

The purpose of the study is to contribute to the existing research that has been conducted in the histological examination of burned bone fragments. To be able to distinguish between human and non-human bone fragments. Through a comparative study of the microstructure of the bones of burned and unburned femoral shafts of humans and various non-human species. Therefore, the aims of the current study were to compare the differences in microstructure morphology of unburned bones from human and non-human species, to determine if prolonged exposure to heat of varying temperatures alters the microstructural morphology of bone, and finally to determine if it is possible to distinguish between burned human and non-human bone using histological methods.

2. EXPERIMENTAL METHOD

For the purpose of this study femur bones from the following species were collected:

- Human (*Homo sapiens sapiens*) (4 femur bones)
- Wildebeest (*Connochaetes gnou*) (5 femur bones)
- Pig (*Sus scrofa*) (5 femur bones)
- Baboon (*Papio ursinus*) (6 femur bones)
- Cow (*Bos taurus*) (3 femur bones)

The exact age of individuals at collection was unknown but all the human samples were obtained from adults. The human bone was obtained from the Department of Human Biology at the University of Cape Town. The porcine and bovine bones were obtained from a local butchery. The wildebeest bones were donated by a local hunter and baboon bones were obtained from the Medical Research Council.

Ethical clearance for the use of human specimens in the study was granted by the Human Research Ethics Committee of the University of Cape Town (HREC: 584/16). No animals were killed for the purpose of the study.

Prior to testing, all bones were cleaned and de-fleshed using a scalpel. The de-fleshed femur bones were then cut into sections using a hacksaw. Three 2cm sections were cut from the mid diaphyseal region of each femur and stored at -20°C until testing commenced. Prior to testing, specimens were thawed at room temperature for 24hrs. It has previously been noted in studies by Lander *et al*. (2014) and Tersigni (2007) that freezing and subsequent thawing of bones has no significant effect on bone microstructure other than qualitative differences relating to pitting. Thus, the freezing and thawing of bone is not expected to effect qualitative measurements taken on the samples in the current study.

2.1. Testing Procedure

The samples from these various specimens were subjected to burning at various temperatures and compared to specimens from an unburned control. These temperatures were chosen because flaming ignition of polyurethane occur around 600°C and roof level temperature can reach 900°C in shack fires (Walls *et al*., 2017).

During testing, one section of each femur was used as a control, one section was burned at 600 °C and the remaining section was burned at 800 °C. All burning experiments were conducted in a muffle furnace (Labofurn, Kiln Contracts Pty Ltd., Cape Town, South Africa). The furnace was heated up to the desired temperature, where upon specimens were placed in the furnace to burn for 20 minutes. After this period the specimens were removed from the furnace and allowed to cool overnight. Before further processing, specimens were embedded in a clear epoxy resin for preservation.

2.2. Thin Section Preparation

Thin sections of all bone specimens were prepared for microscopy using a method similar to the one described by Maat *et al*. (2001) in their paper on manual preparation of ground sections for the microscopy of natural bone tissue.

Unburned bone sections were manually ground down using a combination of waterproof sandpaper (of varying grits), household dishwash liquid soap and water. The bulk of the bone section was ground down on 600grit waterproof sandpaper.

When the bone segment was too thin to grip easily by hand, a smaller piece of waterproof sandpaper was cut and wrapped around a microscope slide to make a gripping tool. The waterproof sandpaper wrapped microscope slide was pressed onto the bone section using minimal force as not to damage the fragile bone section, and the circular grinding motion was continued.

As the bone section become thinner the 600grit waterproof sandpaper was replace with a finer 800 and 1000grit waterproof sandpaper sequentially. Sections were finally polished using 1200grit waterproof sandpaper. Once the section reached a near see-through appearance (˜50µm), it was washed in clear water and placed on absorbent paper to dry. The dried section was mounted onto a microscope slide using Entellan® (Merck) and covered with a large coverslip. The slides were left to dry at least overnight before microscopy was undertaken.

Due to the fragility of the burned specimens, alternative steps were required compared to those described above. Specimens were embedded in epoxy resin for stabilization and preservation. This formed hard plastic blocks which were initially ground down using an automatic belt sander. To obtain smoothness of the sections they were given a final grinding on 1200grit waterproof sandpaper with dishwash liquid soap and water.

2.3. Physical Analysis

Thin sections were viewed under a compound microscope (using an Olympus BX43F) and digital images were collected. Each bone specimen was divided into four quadrants (anterior,

posterior, medial and lateral) and two regions within each quadrant near the periosteal region were sampled for subsequent analysis (Figure 5).

All bone sections were qualitatively analysed for bone structure appearance, such as fractures, presence of carbonation, type of bone present, e.g. lamellar/plexiform bone and visibility of secondary osteons. These qualitative features were analysed by looking under the 10x magnification of the microscope. Bone sections were quantitatively assessed by collecting the following measurements:

- Haversian canal diameter (min and max)
- Haversian canal area
- Haversian system diameter (min and max)
- Haversian system area
- Haversian system density

Measurements were collected from all complete secondary osteons visible in the field of view of the sampling area. Secondary osteons were defined as systems displaying circular lamellar bone around the Haversian canal and encompassed by a well-defined cement line as per White *et al*. (2012).

The minimum and maximum canal diameter was found by using the measuring tool in ImageJ (NIH – National Institute of Health, U.S. Department of Health). Random diameters (lines crossing the centroid of the canal) were measured until the equivalent minimum and maximum were found.

The areas for these parameters were calculated using the formula:

Area = π(0.5 x min diameter) (0.5 x max diameter).

The Haversian system density was calculated as the number of secondary osteons present per mm within the field of view.

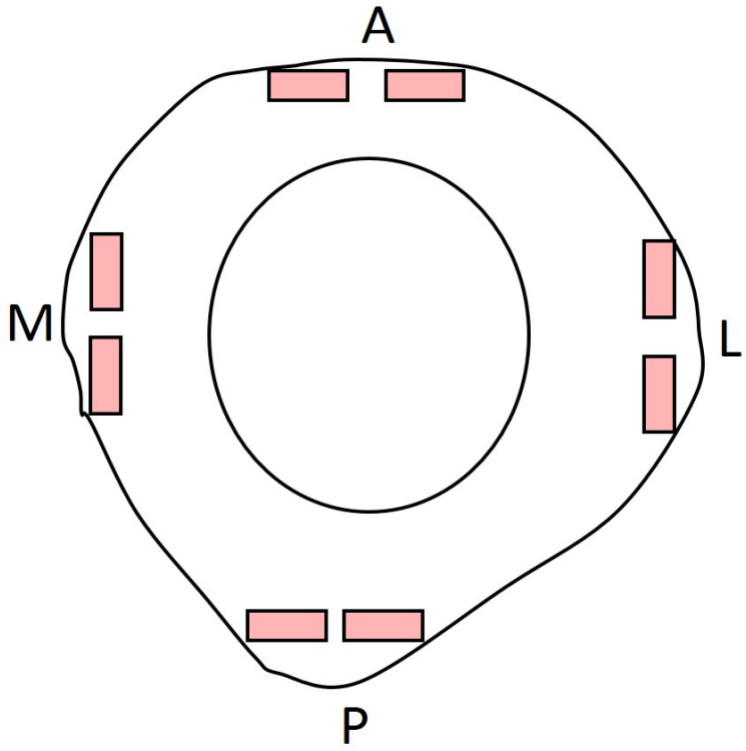


Figure 5 - Quadratic division of femur with pink blocks indicating approximate area of photographs taken for data collection

2.4. Statistical Analysis

Difference in quantitative measures, between the five species were compared using one-way analysis of variance (ANOVA) or the Kruskal Wallis test.

Differences between the unburned bones and those burned at 600°C and 800°C were also analysed using one-way analysis of variance (ANOVA) with repeated measures. Descriptive statistics were used to analyse parameters within a species and difference between quadrants were determined using one-way ANOVA.

All statistical tests were conducted in Stata Ver 14 (Statacorp, NY, USA) and significance of all tests were taken at $\alpha = 0.05$.

3. RESULTS

This study deals with the evaluation of bone sections burned at 600 °C and 800 °C compared to unburned control sections.

3.1. Qualitative Results

A total of five different specimens were analysed. Representative images of each specimen (unburned) can be seen in Figure 6. The overall qualitative aspects of the unburned bone (femur) of the different species can be described as follows:

Human (*Homo sapiens sapiens*) presented with dense secondary osteons in all the quadrants (posterior, anterior, medial and lateral) analysed.

Baboon (*Papio ursinus*) presented with more scattered secondary osteons compared to human bone in the anterior and medial quadrants. In the lateral quadrants scattered secondary osteons with interspersed primary lamellae was noted. The posterior quadrant presented with dense secondary osteons in most of the samples analysed.

Wildebeest (*Connochaetes gnou*) presented with small poorly defined secondary osteons in the posterior quadrant. The anterior and lateral quadrants consisted only of plexiform bone. The medial quadrants presented with some poorly defined secondary osteons interspersed with plexiform bone.

Pig (*Sus scrofa*) bone comprised of plexiform bone in all quadrants analysed in all samples.

Cow (*Bos taurus*) anterior quadrants consisted of plexiform bone only. The lateral and medial quadrants in some samples presented with small secondary osteons in plexiform bone, while in other samples these quadrants only presented as plexiform bone.

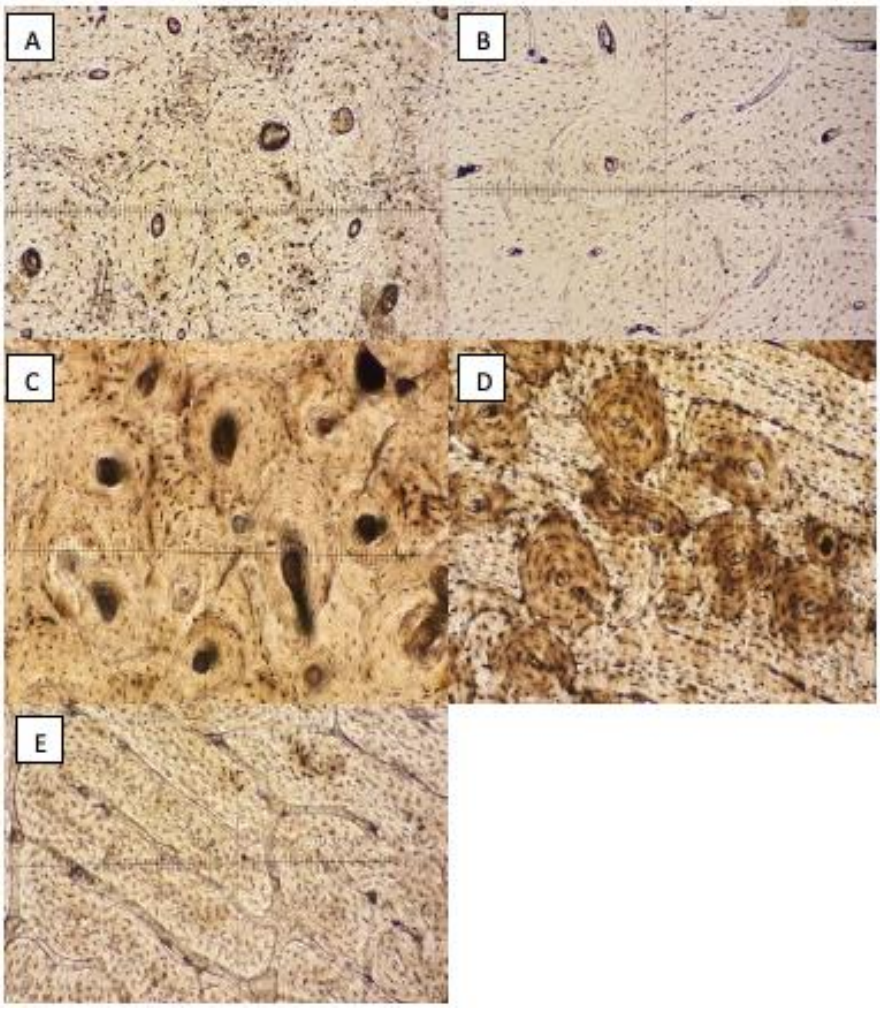


Figure 6 - Different types of unburned bone analysed using an Olympus BX43F microscope. A – Baboon (femur, 40x magnification); B – Cow (femur, 40x magnification); C – Human (femur, 40x magnification); D – Wildebeest (femur, 40x magnification); E – Pig (femur, 40x magnification)

3.2. Quantitative Results

In order to obtain the quantitative results for the unburned and burned samples, 517 slides were analysed.

A total of 523 osteons in unburned bone and 147 in the burned bone samples were analysed to determine the minimum canal diameter, maximum canal diameter, minimum Haversian system diameter, maximum Haversian system diameter, area of the canal and area of Haversian system. These parameters were calculated in each species (Table 1). Differences between species were compared and differences within a species at different temperatures were analysed. Fewer osteons were available for analysis due to carbonation of samples during the burning process and also some samples were lost during the preparation for microscopy due to the brittle nature of the samples after burning.

Porcine specimens were excluded from the quantitative analysis because only plexiform bone was observed in the unburned slides examined.

Table 1 – Comparison between species for different parameters analysed (unburned bone)

Parameter and Species	Number of secondary osteons (N)	Mean (µm)	Median (µm)	SD (µm)	Min (µm)	Max (µm)
Min canal diameter						
Baboon	261	39.24524	34.57	17.22873	10.204	114.269
Human	222	46.17959	42.118	19.69659	9.524	117.955
Cow	7	28.34429	26.723	6.228117	22.289	40.084
Wildebeest	33	26.34436	23.769	11.5482	11.594	69.563
Max canal diameter						
Baboon	261	58.21458	50.889	27.86323	18.031	163.062
Human	222	60.86092	53.9825	28.27307	13.709	197.986
Cow	7	37.24543	40.084	8.816671	25.406	47.031
Wildebeest	33	41.87652	34.203	21.37736	22.638	121.745
Min Haversian system diameter						
Baboon	261	166.6408	157.814	65.3948	47.799	440.071
Human	222	170.5164	164.72	48.44283	79.055	345.351
Cow	7	130.9856	119.155	38.05838	83.012	202.184
Wildebeest	33	163.6031	153.872	44.70621	103.181	337.685
Max Haversian system diameter						
Baboon	261	227.1767	209.182	86.83223	83.451	535.137
Human	222	220.2977	213.075	70.87544	92.933	520.886
Cow	7	162.9009	150.498	31.09813	131.283	206.617
Wildebeest	33	219.64	208.055	56.44673	146.545	460.914
Area of canal						
Baboon	261	2095.705	1400.578	2072.038	144.5041	14634.27
Human	222	2559.31	1791.28	2312.105	154.8836	18341.75
Cow	7	857.127	841.2908	361.9926	444.7508	1452.134
Wildebeest	33	1021.576	626.3211	1194.043	237.894	6651.496
Area of Haversian system						
Baboon	261	33458.44	26440.22	26107.51	3260.531	184120.4
Human	222	31587.22	27849.43	18952.16	6149.869	122161.8
Cow	7	17377.87	13811.14	8236.293	9812.089	32740.98
Wildebeest	33	29818.6	27677.08	18897.28	13262.53	122242.3

Species differences within each parameter were analysed using Kruskal-Wallis tests. These tests revealed that for all parameters analysed there was no significant differences between the species, except within the Haversian canal diameter, the Haversian canal area and the Haversian system area.

The minimum Haversian canal diameter was found to have significant differences between the species ($p = 0.001$). Post hoc testing found that pairwise differences were between human and baboon ($p < 0.001$), wildebeest and human ($p < 0.001$) and wildebeest and baboon ($p = 0.001$). The minimum Haversian canal diameter of human was bigger than that of baboon, wildebeest was smaller than human and wildebeest was smaller than baboon. No significant differences were found between cow and baboon ($p = 0.682$), human ($p = 0.06$), or wildebeest ($p = 1.000$).

The maximum Haversian canal diameter was found to have significant differences between the species ($p = 0.001$). Post hoc testing found that pairwise differences were between wildebeest and baboon ($p = 0.008$) and wildebeest and human ($p = 0.001$). The maximum Haversian canal diameter was bigger in baboon than wildebeest and wildebeest was smaller than baboon. No significant differences were found between cow and baboon ($p = 0.284$), human and baboon ($p = 1.000$), human and cow ($p = 0.155$), or wildebeest and cow ($p = 1.000$).

The minimum Haversian system diameter was found to have no significant differences between the species ($p = 0.001$). Post hoc testing found that there were not pairwise differences between all the species. Cow and baboon ($p = 0.628$), human and baboon ($p = 1.000$), human and cow ($p = 0.437$), wildebeest and baboon ($p = 1.000$), wildebeest and cow ($p = 1.000$) and wildebeest and human ($p = 1.000$).

The maximum Haversian system diameter was found to have no significant differences between the species ($p = 0.001$). Post hoc testing found that there were no significant pairwise differences between all the species, with cow and baboon ($p = 0.195$), human and baboon ($p = 1.000$), human and cow ($p = 0.340$), wildebeest and baboon ($p = 1.000$), wildebeest and cow ($p = 0.492$) and wildebeest and human ($p = 1.000$).

The area of the Haversian canal was found to have significant differences between the species ($p = 0.001$). Post hoc testing found that pairwise differences were between cow and baboon, human and cow, wildebeest and baboon and wildebeest and human with a value of $p < 0.001$. The area of the Haversian canal was smaller in cow than baboon, human was bigger than cow, wildebeest was smaller than baboon and wildebeest was smaller than human. No significant difference was noted between the Haversian canal area of baboons and humans ($p = 0.154$) and wildebeest and cow ($p = 1.000$).

The area of the Haversian system was found to have significant differences between the species ($p = 0.001$). Post hoc testing found that pairwise differences were between cow and baboon ($p < 0.001$), cow and human ($p < 0.001$), wildebeest and baboon ($p < 0.001$) and wildebeest and human ($p < 0.001$). The area of the Haversian system was smaller in cow than baboon, cow was smaller than human, wildebeest was smaller than baboon and wildebeest was smaller than human. No significant difference were found between human and baboon ($p = 1.000$) and wildebeest and cow ($p = 0.739$).

Table 2 – Comparison of osteon density between species

Species	Number of slides measured (N)	Mean	Standard deviation	Minimum	Maximum
Baboon	47	4.465185	2.143768	0.8010065	11.21409
Cow	3	1.869015	0.4624613	1.602013	2.40302
Human	24	7.409311	2.990103	4.005033	17.62214
Wildebeest	12	2.202768	0.9735683	0.8010065	3.204026

Osteon density was calculated by dividing the number of complete secondary osteons in each image by the area of the field of view. The mean osteon density for each species can be seen in Table 2.

The osteon density was found to have significant differences between the species ($p = 0.001$). Post hoc testing found that pairwise difference was between human and baboon ($p < 0.001$), human and cow ($p = 0.001$), wildebeest and baboon ($p = 0.018$) and wildebeest and human

($p < 0.001$). The osteon density was more in human than baboon, human was more than cow, wildebeest was less than baboon and wildebeest was less than human. No significant differences were found between cow and baboon ($p = 0.359$) and wildebeest and cow ($p = 1.000$).

Each specimen was also analysed regarding differences in the parameters in different regions (anterior, posterior, medial, lateral) of the bone.

Parameters were found to significantly differ based on the region of interest within a species. Within baboons the medial quadrant was found to have significantly larger minimum and maximum Haversian canal diameters than the other regions. As a result of this, the Haversian canal areas in the medial region were also significantly larger. The osteon density was, however, significantly greater in the posterior region compared to other quadrants.

Within the cow samples there were no significant differences in any of the parameters between the lateral and medial quadrants. Due to difficulties experienced in cutting the cow bone due to hardness of the bone, there are no data available for the posterior and anterior quadrants.

Within human samples a significant difference was found for minimum and maximum canal diameter when comparing lateral and anterior, posterior and lateral and posterior and medial quadrants. No significant difference was found in the measurements when comparing medial and lateral or posterior and anterior quadrants for these two parameters.

Minimum and maximum Haversian system parameters show a significant difference in size between lateral and anterior and medial and anterior quadrants. No significant difference in size was noted between medial and lateral, posterior and anterior, posterior and lateral and posterior and medial quadrants.

Comparing the area of the canal measurements in human samples, showed that there is a significant difference between lateral and anterior, medial and anterior, posterior and lateral and posterior and medial quadrants. No significant differences were noted between measurements of medial and lateral and posterior and anterior quadrants.

Area of the Haversian system show significant difference in the measurements between the lateral and anterior and medial and anterior quadrants. No significant difference was noted in the measurements of the area of the Haversian system between medial and lateral, posterior and anterior, posterior and lateral and posterior and medial quadrants.

No significant differences were noted in the osteon density in any of the quadrants in the human bone.

Within wildebeest bone samples there are no significant difference in minimum canal diameter, maximum canal diameter, minimum Haversian system diameter, maximum Haversian system diameter and osteon density between any of the quadrants compared. The area of the Haversian system and area of the canal show a similar comparison in measurements, with no significant differences between lateral and anterior, medial and anterior and medial and lateral quadrants. A significant difference in measurements are noted between posterior and anterior, posterior and lateral and posterior and medial quadrants for these two parameters.

3.3 Effects of Temperature on Bone Histomorphometry

To determine if significant change occurs in microstructure size as a result of an increase in temperature, a repeated measures ANOVA was performed on each parameter within a species. This test found overall significance ($p < 0.0001$) for all parameters, suggesting that temperature does affect the size of microstructures, although results may be skewed due to difficulty in measuring structures that were highly carbonised, particularly those burned at 600°C.

Exposure to heat, regardless of the temperature, resulted in changes to the microstructure of bone. No difference was noted between the samples of the same species burned at 600°C compared to samples burned at 800°C.

Qualitatively, the bone burned at 600°C had a black appearance and the bone burned at 800°C had a greyish appearance. Human bone burned at 600°C presented with severe carbonation with no secondary osteons visible in some parts of most quadrants analysed, which made data collection very limited in some quadrants. Human bone burned at 800°C presented with secondary osteons more visible but were difficult to measure due to few clear cement lines

in certain samples and quadrants. Baboon bone burned at 600°C presented with severe carbonation in most samples, which made measurement of secondary osteons near impossible. Baboon bone burned at 800°C presented with scattered secondary osteons visible but due to few clear cement lines present the measurements were also limited.

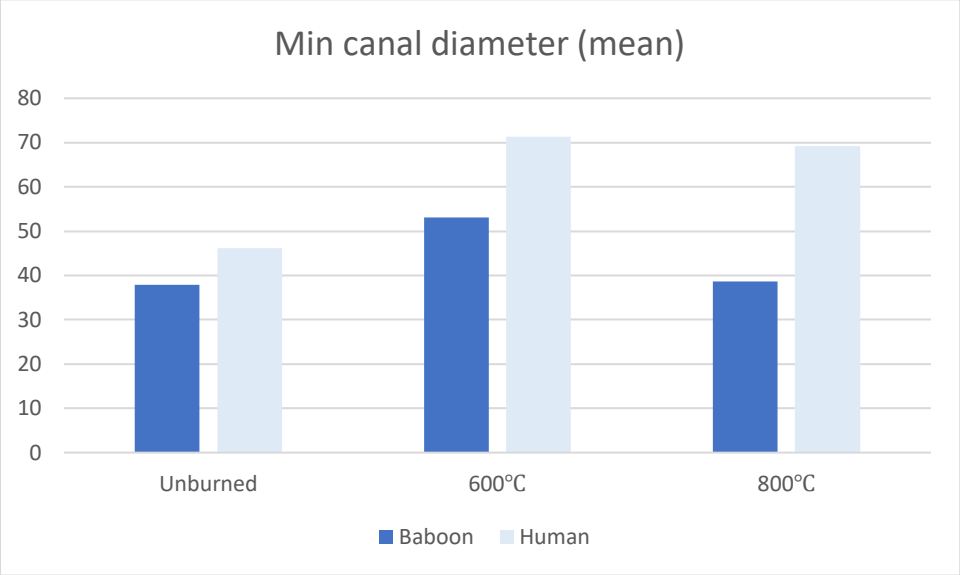
The carbonization and fragility of cow and wildebeest bones resulted in almost no collectable data; therefore, these specimens were removed from analysis in this section.

Table 3 – Comparison of parameters in unburned bone and bone burned at 600°C and 800°C

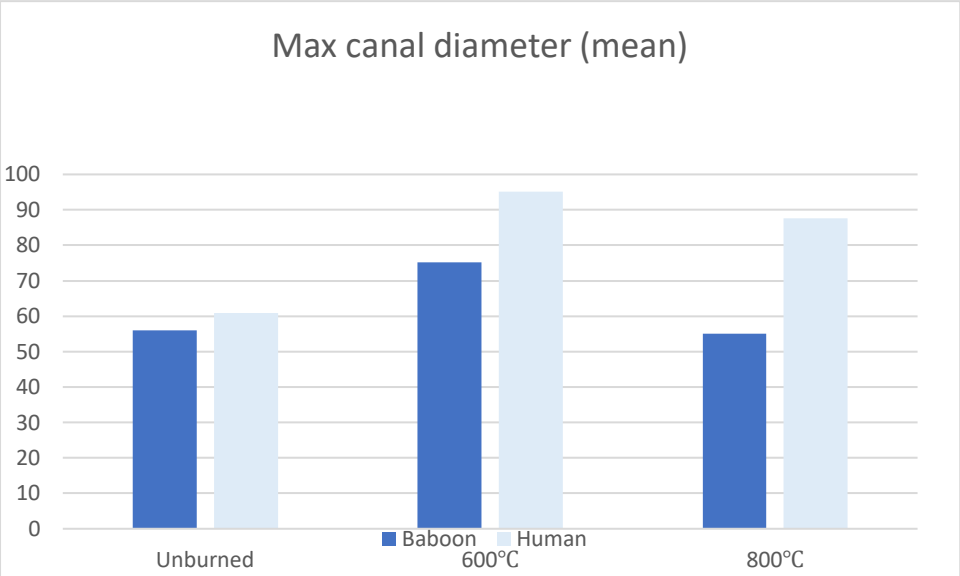
	Unburned			600 °C			800 °C		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Min canal diameter									
Baboon	198	37.960727	13.2881	16	53.151312##	13.58378	20	38.67795	15.43653
Human	222	46.179586	19.69659	61	71.288295##	26.57895	50	69.27342#	33.64897
Max canal diameter									
Baboon	198	56.004051	22.5922	16	75.225687##	34.89761	20	54.99065#	16.68063
Human	222	60.860923	28.27307	61	95.182836##	35.50511	50	87.60062#	39.75852
Min Haversian system diameter									
Baboon	198	156.90523	57.46678	16	151.20437	31.15729	20	166.55885	34.1286
Human	222	170.5164	48.44283	61	202.66539#	45.3999	50	221.51254#	59.31756
Max Haversian system diameter									
Baboon	198	212.95603	78.27756	16	197.61469	45.06105	20	250.98225	57.11984
Human	222	220.29769	70.87544	61	267.62993#	69.28726	50	265.14142	78.76056
Area of canal									
Baboon	198	1839.9537	1448.885	16	3383.9821#	2287.643	20	1663.0829	1208.416
Human	222	2559.3096	2312.105	61	5927.2657##	4160.739	50	5734.5535#	5488.551
Area of Haversian system									
Baboon	198	29210.612	20349.47	16	24175.408	9466.078	20	33452.84	14385.49
Human	222	31587.216	18952.16	61	44253.183	20745.28	50	49094.334#	26957.62

 # Significantly different from unburned at 0.05 level
Significantly different from unburned at 0.001 level

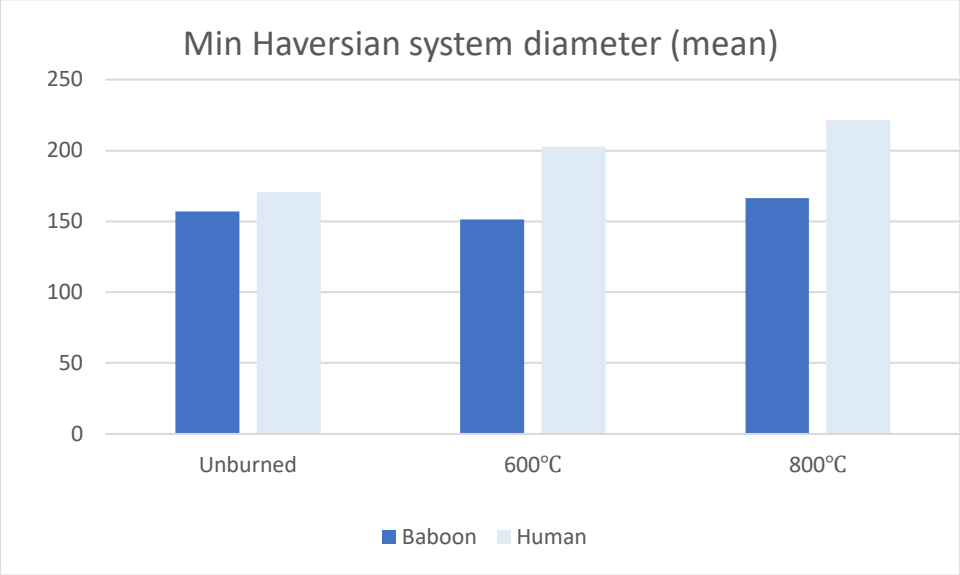
Graph 1 – Minimum canal diameter (mean)



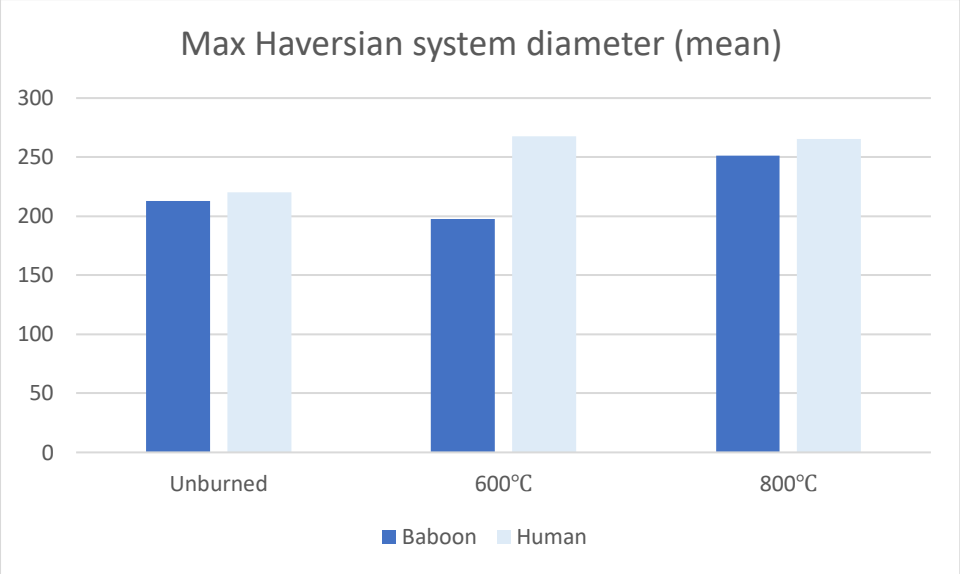
Graph 2 – Maximum canal diameter (mean)



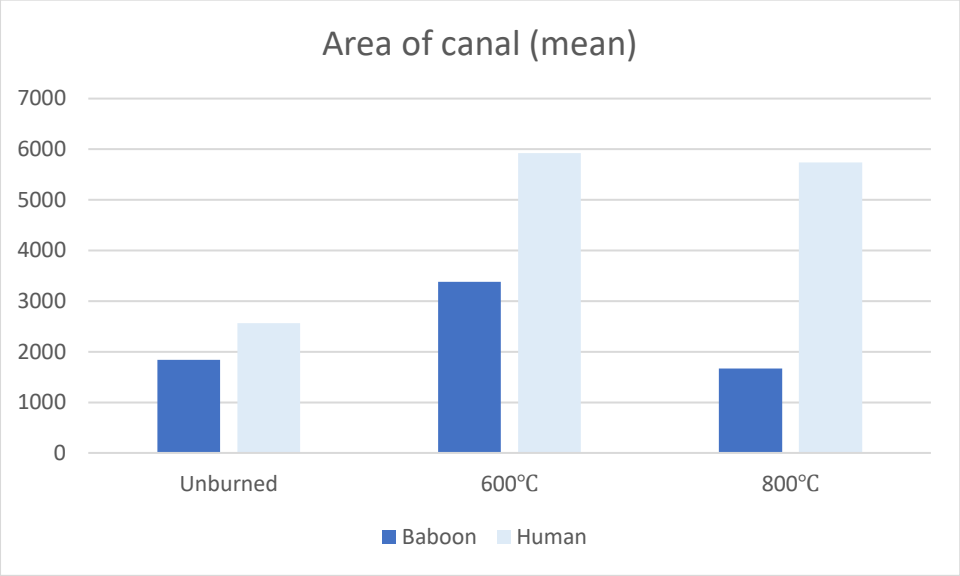
Graph 3 – Minimum Haversian system diameter (mean)



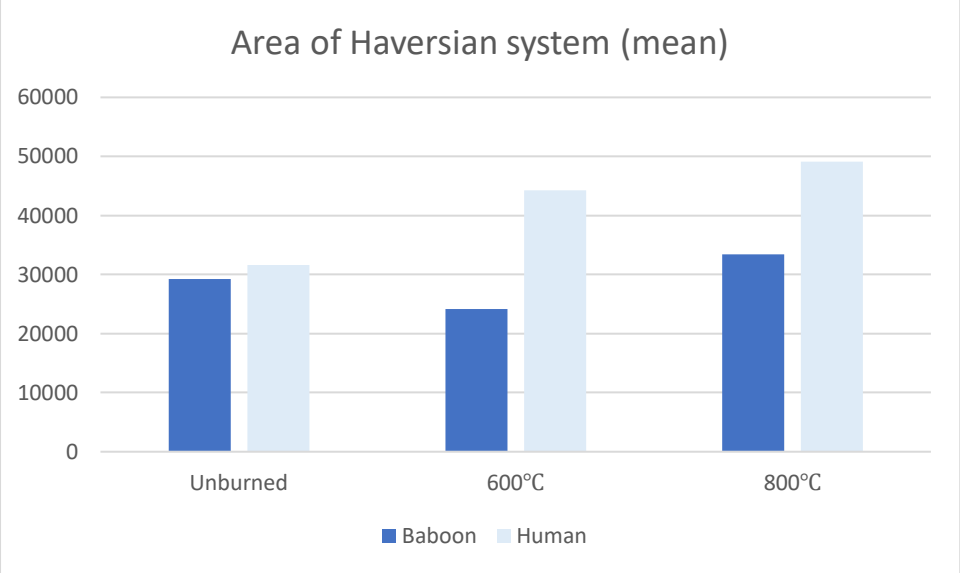
Graph 4 – Maximum Haversian system diameter (mean)



Graph 5 – Area of canal (mean)



Graph 6 – Area of Haversian system (mean)



Wilcoxon signed rank tests were applied to the data in Table 3 and showed that the minimum canal diameter of baboon and human bone burned at 600°C is significantly different from unburned at the 0.001 level. Human bone burned at 800°C is significantly different from unburned at 0.05 level.

Maximum canal diameter of baboon and human bone burned at 600°C is significantly different from unburned at 0.001 level. Both baboon and human bone burned at 800°C are significantly different from unburned at 0.05 level.

The minimum Haversian system diameter for human bone burned at both 600°C and 800°C are significantly different from unburned at 0.05 level.

Maximum Haversian system diameter of human bone burned at 600°C are significantly different from unburned at 0.05 level.

Area of the canal of baboon bone burned at 600°C are significantly different from unburned at 0.05 level. Area of the canal of human bone burned at 600°C are significantly different from unburned at 0.001 level and human bone burned at 800°C are significantly different from unburned at 0.05 level.

Area of the Haversian system of human bone burned at 800°C are significantly different at 0.05 level.

All the other parameters and temperature comparisons between unburned and burned at 600°C and 800°C not mentioned above are not significantly different at either of the levels.

The data in Table 3 shows that there was a difference at all temperatures compared to unburned bone, however there was no significant differences between 600°C and 800°C.

4. DISCUSSION

Bodies are burned in a number of different circumstances which may be deliberate (homicide or in an attempt to destroy evidence) or accidental (industrial/ household fires, aircraft and road traffic accidents). Archeological bone samples may also present with evidence of burning. Burned bone can present as highly fragmented and may be comingled with debris and rubbish (which may include bones of non-human origin). Identification of the fragments as being human in origin can therefore be a difficult task (Ubelaker, 2009).

Extended exposure of the human body to fire, leads to severe alterations especially in the head of the body. The alterations make it difficult to determine the cause of death and if a criminal element was involved. If the person was alive when the fire was started, the pathologist will see presence of soot in the airways, oesophagus and stomach on autopsy. Positive levels of carbon monoxide or cyanide may also be found depending on the fuel used and the nature of the fire (Fanton *et al*., 2006).

The aim of the current study was to determine if it was possible to differentiate animal and human bones in a burned state. However future research may be needed to ascertain the effect of age on such analyses, particularly with regard to juvenile human bone which may more closely resemble the histomorphometrics of baboon bone.

The current study investigated the differences in micromorphology and morphometry between different animal species and the effect of temperature on these structures. Previous research has demonstrated that it is possible to differentiate certain animals based on micromorphometry (Hillier & Bell, 2007). However, limited research has investigated the effect temperature may have on micromorphology and morphometry of different species and what effect this may play on the ability to differentiate human from non-human bone.

4.1. Histological Comparison of Unburned Bone

The initial focus of this study was to describe the micromorphology and morphometry of bone specimens which had not undergone any form of trauma or burning. The morphometric data presented was similar to that seen in other studies (Martiniakova *et al*., 2007). Human bone can easily be differentiated from pig, cow or wildebeest bone due to only a few or even no osteons being present in the bones of those animals. Cow and wildebeest presented with only a few osteons in some parts of the bone, while pig bone in the current sample consisted almost entirely of plexiform bone.

Baboon and human bone were, however, very similar in appearance on a microscopic level regarding amount and size of osteons observed. The current study demonstrated that the morphometry of Haversian canals in human bone were typically larger than in the baboon specimens in an unburned state. There was however, considerable overlap between these two species and the micromorphometric differences were not considered significant. Similar findings were noted by Brits *et al*., (2014) when evaluating various animals such as cows, sheep, pigs and non-human primates. They found that cow and pig femora consisted mainly of plexiform bone and non-human primates exhibited medium sized osteons with large central Haversian canals.

African species such as wildebeest have not previously been used as specimens in a similar research context. Wildebeest are mostly found in the north-western, north-eastern, eastern and northern parts of South Africa, which constitute a large part of South Africa. This makes it is of great value to have included the species in the current study. The current study demonstrated wildebeest bone to be somewhat similar to cow bone, exhibiting mainly plexiform bone with only a few osteons present in some parts of the bone. Morphometrically there was some overlap between these two specimens, however, specimens with larger micro-parameters were more likely to be of wildebeest origin.

The presence or absence of osteon banding is another factor which can be used to differentiate species histologically. Osteon banding is the alignment of Haversian systems into an ordered, regular row or band. Mulhern *et al*., (2001) conducted histological examinations of femoral bone samples of human, sheep and miniature pigs. Osteon banding was noted to some degree in all samples but were rare in human and sheep samples analysed. If present in human samples the osteon banding consisted of a short, isolated band within lamellar bone. In the current study osteon banding was noted in two of the five porcine samples. Mulhern *et al*., (2001) found osteon bands in the posterior quadrant, towards the edge of the endosteal region of the femur bone of the miniature swine studied. It consisted of five or more primary osteons surrounded by plexiform bone (Mulhern & Ubelaker, 2001). During a study on human, dog, badger and pig cranial bone conducted by Simmons *et al*., (2016), osteon banding was also found. The bands were typically less than five osteons long in humans, however it is noted that the use of osteon banding in cranial bone for species identification may be somewhat flawed due to the inconsistent observation of Haversian systems Simmons *et al*., (2016). Some human bones can also present with osteon banding Andronowski *et al*., (2017).

4.2. Macroscopic Effect of Temperature on Bone

Specimens in the current study were subjected to burning at 600°C or 800°C for 20 minutes to establish the effect of temperature on the macro and microstructures of bone. These temperatures were chosen as they represent temperatures obtained by general house fires in an enclosed space [600°C - 800°C] (DeHaan, 2015) as well as fires in informal settlements [800°C - 1000°C] (Walls, Olivier & Eksteen, 2017).

Forensic scientists or investigators may be confronted with deliberately or accidentally burned human remains in various settings. The setting of a fire including type of fuel, fuel load, duration and intensity of fire all play a role in fire temperature and subsequent heat alterations to bone. It is therefore important to conduct research under a variety of conditions.

Shipman *et al*., (1984) described colour, morphology, crystal structure and shrinkage changes observed during a study conducted on burned bones and teeth. They observed similar changes in bone colour as what was noted in the current study, with bones burned at 600°C exhibiting a black appearance and light grey with blueish black centres at 800°C.

Colour is not the only macroscopic change as a result of heat exposure. Bones can also change size and shape (Bohnert *et al*., 1998; Ellingham *et al*., 2015; Shipman *et al*., 1984; Thompson, 2004, 2005). These changes are believed to be a result of warping, recrystallization and water loss, although as noted by Thompson (2005), the size change may be positive or negative in nature. At lower burning temperatures (<700°C) some bones exhibited increased dimensions (Thompson, 2005). Such changes in dimension and shape of bone will have consequences on any subsequent anthropological analysis requiring shape and size (sex, ancestry, stature). The macroscopic effects of heat exposure were not within the scope of the study and therefore not recorded. It is only discussed here to highlight that with such changes occurring at the macroscopic level, underlying changes must also be occurring at microscopic level. There has however, been far less research analysing this aspect. There is a clear disparity in studies conducted at the macroscopic level compared to the microscopic level.

4.3. Microscopic Effect of Temperature on Bone

Increased temperature as a result of burning can have several effects on the microstructure of bone. These changes are primarily as a result of warping, dimensional changes and fracturing. As noted in the current study, the main effect at the microscopic level was an increase in the diameter of Haversian canals and subsequently a significant increase in canal area.

Absolonova *et al*. (2013) did a study to estimate age of death from burned skeletal remains using ribs. After heat application it was found that most microstructures were unidentifiable

except for intact osteons and Haversian canals. After comparing it with the current study it is evident that there are challenges in identifying burned bone regardless of the type of bone used.

Nelson (1992) did a study on femoral bone sections, which were burned in a propane-fired kiln between 1000 - 1500°F for 30 minutes. It was found that the Haversian canal size markedly increases in many instances by up to 10.5% with an associated decrease in the osteon diameter through the burning process. The canal size can possibly increase in size due to the burning away of soft tissue in the Haversian canal (Nelson, 1992). In the current study it was found that the minimum canal diameter of the baboon samples increased by 33.34% at 600°C, but only increased by 1.87% at 800°C in comparison with the unburned bone. The human minimum canal diameter increased by 42.75% at 600°C and increased by 40.01% at 800°C. The maximum canal diameter of the baboon samples increased by 29.30% at 600°C and showed a decrease of 1.83% at 800°C. The maximum canal diameter of the human samples increased by 43.99% at 600°C and increased by 36.02% at 800°C.

Thompson (2005) found that warping of bone is the most prominent artefact of burning, especially in bone which were fleshed at the time of burning, due to the contraction of muscle fibres. Warping was not seen in the current study because de-fleshed bone samples were used. Another observation made by Thompson (2005) was that the dimensions of the bone changes were due to expansion of air in the medullary cavity. In the current study the femur bones were cut into discs and the contents of the medullary cavity removed before heating. The dimension of the bone samples was also not one of the measurable parameters studied. Hanson & Cain (2007) mentioned another very important change seen microscopically when heat around 600°C is applied to bone samples, which are cracks throughout the matrix of the bone sample. In the current study there were cracks observed in the matrix of the bone samples of both human and non-human at both 600°C and 800°C.

It is clear from these results that temperature does affect the microstructure of bone. It must, however, be noted that there was a substantial decrease in amount of measurable canals in the burned samples compared to the unburned samples.

4.4. Differentiating Human from Non-Human Burnt Bone

It is a well-established fact that different species of animals can be differentiated from humans on a histological level through examining their skeletal remains in an unburned state. Fewer studies have however been done to differentiate the bones from different species in a burned state.

In the current study femur bones were used due to the uniformity and alignment of microstructures, unlike in the cranium, as observed by Simmons *et al*., (2016) during their study using cranial bones. Simmons *et al*., (2016) did a study to assess species-specific variation in cranial bones in an unburned and burned state. Histomorphology of cranial bones were found to be unreliable using osteon banding, because of inconsistent observations of Haversian systems. In this study it was also found that osteon banding in non-human bone sections are better aligned than those seen in human sections. This study suggested that a lack of orientated stress load lines in the cranial vault bone, results in a lower alignment of osteons compared to that found in long bones. Because of this the histology of cranial bones appears less organized than that of long bones, leading to difficulties in interpretation of species-related differences. It was noted in their study that secondary osteons presented multiple orientations across all species, except the sagittal crest area of dog bone in transverse sections. This study further showed that cancellous bone sections often appear opaque, which makes histological morphology interpretation difficult. Other heat induced effects apart from that cancellous bone are more readily destroyed than robust compact cortical bone, are black deposits, granularity and micro fractures (Simmons *et al*., 2016).

In the current study histological methods were used to determine if human and non-human bones can be identified in a burned state, using parameters regarding the Haversian canal, such as the area and minimum and maximum diameters. It was found that burning the bone samples causes carbonation and brittleness of the samples. The brittleness was partially overcome by imbedding the samples in resin, but that lead to difficulties in sanding the samples down sufficiently to get clear photos under the microscope, due to thickness of samples and carbonation present. This led to fewer osteons being measured to determine the Haversian canal parameters. It was however, found that it will be possible to distinguish between human and baboon bones in a burned state, as the baboon Haversian canal

parameters are smaller than the human ones (see Table 3). Cattaneo *et al*., (1999) did a study to determine the human origin of fragments of burned bone using a comparative study of different methods – histological, immunological and DNA. The failure of immunological and DNA methods to produce reliable results in forensic cases where burned bones were involved has made it clear that histological methods using quantitative microscopy for identification of burned bone samples have not become redundant. It was found that the most important discriminating factor was the Haversian canal, specifically the area and minimum and maximum diameters. The morphological examination in this study was carried out by two independent observers – a zoologist and an anthropologist (Cattaneo, *et al*., 1999).

In addition, the use of DNA and immunology techniques will be more costly than histological techniques, which are relatively inexpensive. Thus, histological methods will be more easily accessible than these methods.

4.5. Limitations

The process of making slides from both the burned and unburned bone is a very labour intensive, but it is a cost-effective method which does not require expensive equipment. The quality of the slide can be compromised if the bone could not be sanded down enough to produce a sufficiently thin slide. This is typically not a problem which is encountered when processing unburned bone. However, burned bone can be very brittle and fragile making processing in the same manner as unburned bone difficult. To stabilize the bone, it was embedded into clear epoxy resin. The hardness of the resin makes manual grinding difficult and an electric belt sander was used to make the resin embedded samples as thin as possible without the samples starting to break up. Samples were still not able to be ground to a sufficient thickness for viewing with a light microscope and additional external light sources had to be used to illuminate the samples enough for viewing and quantitative measurements. High temperature burning lead to no visible features other than haversian canals, other methodologies may therefore be necessary such as fourier transform infrared spectrometry or SEM-EDX (scanning electron microscopy coupled with electron dispersive X-ray spectroscopy) to analyse the microconstituents of bone. Future research would benefit from the inclusion of reflected light microscopy and SEM analysis.

The fragility of bones also created limitations regarding sample size. This was a problem especially with the burned samples, where due to fragility of the samples, some samples were lost. Very basic tools were used during the entire process to ensure that it remained cost-effective. It is possible that with more specialized tools the process may be less time-consuming, and should be considered for future studies.

The burning process was conducted in an electric furnace, which is a controlled environment. In the forensic setting burned remains are usually the result of a house, car, bush or a fire started by an arsonist. In these settings there are several different factors which play a role in the burning process. Factors include accelerant used, other materials surrounding the body such as wood, paper or plastic and the temperature reached by the fire. Due to these differences in environments it is necessary to investigate and establish basic experimental data where temperature can be accurately controlled and burning evaluated, which can be used to aid in the evaluation of more complex situations in future studies.

Limited research has been conducted investigating the burning of fleshed specimens (Ellingham *et al*., 2016). While in reality bones are generally fleshed, this study utilised defleshed bones. The purpose of this study was to analyse in a controlled environment the effect of temperature on the microstructure of bone. Bones had to be defleshed in order to maintain the matched type analysis of using the same specimens at different temperatures. This is in accordance with the literature (Ubelaker, 2009).

5. CONCLUSION

In conclusion, the current study demonstrated the ability to produce quality histological slides of burned and unburned bone with relatively inexpensive equipment. This is however a labour-intensive process, which needs to be taken into consideration when selecting this technique over others. The burned bone samples were more fragile, but in most cases, it was still possible to analyse and distinguish it as either human or animal. The samples burned at 600°C were more difficult to analyse due to severe carbonization.

Burning increased the difficulty associated with differentiation due to the presence of carbonization and fragmentation of bone, however for most animals used in the study it is possible to differentiate between species burned at 600°C and 800°C utilizing histological

techniques. Baboon specimens were, however, very similar to humans and differentiation was difficult. It was further noted that histological structures may acquire heat induced alterations to the size of micro-structures further complicating species differentiation between these two species.

This is very useful information when analysing forensic samples obtained from unknown source. The other animals used in this study were easily distinguished from human samples due to the presence of mainly plexiform bone with very few if any secondary osteons.

6. REFERENCES

Absolonova, K., Veleminsky, P., Dobisikova, M., Beran, M., & Zocova, J. (2013). Histological Estimation of Age at Death from the Compact Bone of Burned and Unburned Human Ribs. *Journal of Forensic Sciences,* 58(S1), S135-S145.

Andronowski, J., Pratt, I., & Cooper, D. (2017). Occurance of osteon banding in adult human cortical bone. *American Journal of Physical Anthropology, 164*(3), 635-642.

Beckett, S., Rogers, K., & Clement, J. G. (2011, May). Inter-Species Variation in Bone Mineral Behaviour upon Heating. *Journal of Forensic Sciences, 56*(3), 571-579.

Bohnert, M., Rost, T., Pollak, S. (1998). The degree of destruction of human bodies in relation to the duration of the fire. Forensic Science International, 95(1), 11-21

Brits, D., Steyn, M., & Noelle L'Abbe, E. (2014). A histomorphological analysis of human and non-human femora. *International Journal of Legal Medicine,* 128(2), 369-377.

Cattaneo, C., DiMartino, S., Scali, S., Craig, O., Grandi, M., & Sokol, R. (1999). Determining the human origin of fragments of burnt bone: a comparative study of histological, immunological and DNA techniques. *Forensic Sciences International, 102*, 181-191.

Cattaneo, C., Porta, D., Gibelli, D., & Gamba, C. (2009). Histological Determination of the Human Origin of Bone Fragments. *Journal of Forensic Sciences, 54*(3), 531-533.

DeHaan, J. D. (2015). Fire and Bodies - Chapter 1. In C. W. Schmidt, & S. A. Symes, *The Analysis of Burned Human Remains.* Academic Press: London.

Ellingham, S.T., Thompson, T.J., Islam, M., & Gillian, T. (2015). Estimating temperature exposure of burnt bone - A methodological review. *Science and Justice*, *55* (3), 181 - 188.

Fanton, L., Jdeed, K., Tilhet-Coartet, S., & Malicier, D. (2006). Criminal burning. *Forensic Science International, 158*, 87-93.

Hanson, M., & Cain, C. R. (2007). Examining histology to identify burned bone. *Journal of Archeological Sciences, 34*, 1902-1913.

Hillier, M. L., & Bell, L. S. (2007). Differentiating Human Bone from Animal Bone: A Review of Histological Methods. *Journal of Forensic Sciences, 52*(2), 249-263.

Lander, S., Brits, D., & Hosie, M. (2014). The effects of freezing, boiling and degreasing on the microstructure of bone. *HOMO - Journal of Comparative Human Biology*, *65* (2), 131-142.

Maat, G. J., Van den Bos, R. P., & Aarents, M. (2001). Manual Preparation of Ground Sections for the Microscopy of Natural Bone Tissue: Update and

Martiniakova, M., Grosskopf, B., Olmeka, R., Dammers, K., Vondrakova, M., & Bauerova, M. (2007). Histological Study of Compact Bone Tissue in Some Mammals: A Method for Species Determination. *17*(1), 82-90.

Modification of Frost's "Rapid Manual Method". *International Journal of Osteoarcheology*, *11* (5), 366-374.

Mohd Nor, F., Pastor, R. F., & Schutkowski, H. (2014). Age at death estimation from bone histology in Malasian males. *Medicine, Science and the Law, 54*(4), 203-208.

Mulhern, D. M., & Ubelaker, D. H. (2001). Differences in Osteon Banding Between Human and Nonhuman Bone. *Journal of Forensic Science, 46(2)*, 220-222.

Mulhern, D. M., & Ubelaker, D. H. (2003). Histologic Examination of Bone Development in Juvenile Chimpanzees. *American Journal of Physical Anthroplogy: The official publication of the American assosiation of physical anthropologist, 122*(2), 127-134.

Nelson, R. (1992). A Microscopic Comparison of Fresh and Burned Bone. *Journal of Forensic Science, 37* (4), 1055-1060.

Owsley, DW., Mires, AM., & Keith, MS. (1985). Case involving differentiation of deer and human bone fragments. *Journal of Forensic Science*, 30(2), 572-578

Shipman, P., Foster, G., & Schoeninger, M. (1984). Burnt Bones and Teeth: an Experimental Study of Color, Morphology, Crystal Structure and Shrinkage. *Journal of Archeological Sciences, 11*, 307-325.

Simmons, T., Goodburn, B., & Singhrao, S. (2016). Decision tree analysis as a supplementary tool to enhance histomorphologicl differentiation when distinguishing human from non-human cranial bone in both burnt and unburnt states: A feasibility study. *56(I)*, 36-45.

Tersigni, M. A. (2007). Frozen Human Bone: A Microscopic Investigation. *Journal of Forensic Sciences, 52*(1), 16-20.

Thompson, T. (2004). Recent advances in the study of burned bone and their implications for forensic anthropology. S203 - S205.

Thompson, T. (2005). Heat-induced Dimensional Changes in Bone and their Consequences for Forensic Anthropology. *Journal of Forensic Science, 50*(5), 1-8.

Ubelaker, D. H. (2009). The forensic evaluation of burned skeletal remains: A synthesis. *Forensic Science International*, 1-5.

Walls, R., Olivier, G., & Eksteen, R. (2017). Informal settlement fires in South Africa: Fire engineering overview and ful-scale tests on "shacks". *Fire Safety Journal, 91*, 997-1006.

White, T. D., Black, M. T., & Folkens, P. A. (2012). *Human Osteology* (Third ed.). Elsevier Academic Press.

APPENDIX A: Tables for regional breakdown of parameters in unburned bone

A1 – Regional breakdown of parameters in unburned baboon bone.

Variable	Anterior Quadrant		Lateral Quadrant		Medial Quadrant		Posterior Quadrant	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Min canal diameter	39.64062	15.82155	37.3183	18.66692	48.39085	19.43635	33.93254	13.03915
Max canal diameter	54.8241	20.98523	57.37136	31.4004	72.79487	34.43073	51.11139	20.85776
Min Haversian system diameter	152.1888	52.73403	140.1689	79.95608	182.0274	76.09063	178.6454	50.03725
Max Haversian system diameter	207.5908	67.05136	187.3034	83.75333	246.306	108.123	246.581	74.28791
Area of canal	1931.458	1854.708	2013.586	2187.54	3153.695	2782.65	1518.864	1158.308
Area of Haversian system	27125.98	17656.5	24720.22	28864.83	40714.12	35065.67	36686.18	19720.32
Density	3.871532	1.401328	3.422482	1.245606	4.138534	2.044553	6.341302	2.497252

A2 – Regional breakdown of parameters in unburned cow bone. (No reportable observations made in the posterior and anterior quadrants)

Variable	Lateral Quadrant		Medial Quadrant	
	Mean	SD	Mean	SD
Min canal diameter	31.92633	7.742332	25.65775	3.893847
Max canal diameter	37.28333	10.52625	37.217	9.033104
Min Haversian system diameter	155.6377	42.41947	112.4965	25.17444
Max Haversian system diameter	171.3187	30.84573	156.5875	34.29107
Area of canal	365.6927	565.588	384.2935	453.3006
Area of Haversian system	8108.43	12376.23	7097.204	8617.411
Density	2.40302	-	1.602013	0

A3 - Regional breakdown of parameters in unburned human bone.

Variable	Anterior Quadrant		Lateral Quadrant		Medial Quadrant		Posterior Quadrant	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Min canal diameter	52.96165	17.68884	36.00093	14.84233	39.35643	11.66785	54.10966	23.70564
Max canal diameter	70.37102	30.28653	46.53952	19.60702	53.16667	20.43997	70.64972	31.22514
Min Haversian system diameter	189.7102	56.64352	154.9326	43.97023	160.0267	39.96155	175.8513	45.44366
Max Haversian system diameter	248.2962	79.52936	196.7838	63.85095	205.2865	64.73915	228.4974	65.61646
Area of canal	3243.313	2260.642	1506.573	1219.688	1765.911	1164.45	3457.163	3034.73
Area of Haversian system	39686.78	23314.53	25590.9	14025.25	27405.04	15298.1	33192.44	18916.13
Density	6.942057	1.491393	10.81359	4.738819	6.541553	2.839528	6.70843	2.094795

A4 - Regional breakdown of parameters in unburned wildebeest bone.

Variable	Anterior Quadrant		Lateral Quadrant		Medial Quadrant		Posterior Quadrant	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Min canal diameter	25.4435	5.980867	33.256	-	21.4939	4.888133	28.85528	14.44141
Max canal diameter	34.782	6.315779	35.113	-	32.4827	7.021837	49.04761	26.57415
Min Haversian system diameter	156.5188	47.01596	242.506	-	143.076	25.0542	172.1979	48.32856
Max Haversian system diameter	231.216	46.25335	260.68	-	181.5538	26.59011	235.9465	63.09001
Area of canal	199.3541	345.5903	76.42696	264.7508	291.3098	317.194	1223.453	1517.491
Area of Haversian system	8277.826	14694.35	4137.508	14332.75	10945.5	11588.52	30525.48	24287.99
Density	1.602013	0	0.8010065	-	2.670022	0.9249227	2.40302	1.013202

APPENDIX B: Ethics certificate

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
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Website: www.health.uct.ac.za/fhs/research/humanethics/forms

9 September 2016

HREC REF: 584/2016

Mr C Mole
Pathology
Forensic Medicine and Toxicology
Entrance 2, level 5
Falmouth Building

Dear Mr Mole

PROJECT TITLE: THE USE OF HISTOLOGICAL METHODS TO DISTINGUISH BETWEEN THE BURNED REMAINS OF HUMAN AND NON-HUMAN BONE (MPhil-candidate-Ms L Labuschagne)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th September 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

The HREC acknowledge that the MPhil student Lizl Labuschagne will also be involved in this study.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.

HREC 584/2016

APPENDIX C: Turnitin certificate

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